# APOA1 GENE POLYMORPHISM G–75A (rs 1799837) AND C+83T (rs 5069) AND ITS ASSOCIATION WITH CORONARY ARTERY DISEASE

*Dissertation submitted to*
**The Tamilnadu Dr.MGR Medical University**
*In partial fulfillment of the regulations for*
*the award of the degree of*

**M.D.BIOCHEMISTRY**
**Branch XIII**

**DEPARTMENT OF BIOCHEMISTRY**
**KILPAUK MEDICAL COLLEGE**
**CHENNAI-600010.**
**THE TAMILNADU DR.MGR MEDICAL UNIVERSITY**
**CHENNAI-600032**

**APRIL-2015**

# CERTIFICATE

This to certify that the dissertation entitled **"APOA1 GENE POLYMORPHISM G–75A(rs1799837) AND C+83T (rs 5069) AND ITS ASSOCIATION WITH CORONARY ARTERY DISEASE"** by the candidate **DR.G.UDAYA KUMARI** for **M.D Biochemistry (Branch XIII)** is a bonafide record of the research done by her during the period of study **(2012 –2015)** in the Department of Biochemistry, Kilpauk Medical College, Chennai – 600010.

**Dr.N.GUNASEKARAN., M.D., DTCD.**
**DEAN,**
Kilpauk Medical College,
Chennai – 600010.

**Dr.R.LALITHA, M.D.,**
**PROFESSOR & HEAD,**
Department of Biochemistry,
Kilpauk Medical College,
Chennai – 600010

Date :

Station :

# DECLARATION

I solemnly declare that this dissertation entitled **"APOA1 GENE POLYMORPHISM G–75A(rs 1799837) AND C+83T (rs 5069)AND ITS ASSOCIATION WITH CORONARY ARTERY DISEASE"** was written by me in the Department of Biochemistry, Kilpauk Medical College, Chennai, under the guidance and supervision of Biochemistry **Prof.V.MEERA,M.D.,** Associate Professor, Department of Biochemistry & Kilpauk Medical College, Chennai – 600010. This dissertation is submitted to **THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY** Chennai, in partial fulfillment of the university regulations for the award of **DEGREE OF M.D BIOCHEMISTRY (BRANCH-XIII)** examinations to be held in **APRIL – 2015.**

**Signature of the Guide**

**Dr.G.UDAYA KUMARI**

**Date :**

**Place : Chennai**

# ACKNOWLEDGEMENT

**"Gratitude is the humble gift, I can give to my beloved Teachers".** I express my profound gratitude to the **Dean Dr.N.Gunasekaran M.D**., Kilpauk Medical College and Hospital, Chennai for granting me permission to conduct the study at the Department of Biochemistry and Department of Cardiology, Kilpauk Medical College and Hospital.

I thank my respectful **Prof. R.Lalitha,M.D., HOD,** Department of Biochemistry, Kilpauk Medical College& Hospital, for having been very much supportive and encouraging for conducting this study.

I express my sincere thanks to Former **Dean, Dr.P.Ramakrishnan, M.D., & Prof. Dr. R.Nagendran., M.D.,** former HOD of Biochemistry for having been very much supportive and encouraging for conducting this study.

I express my sincere thanks to **Prof.G.Gnanavelu.,M.D.,D.M., Former HOD &Prof.R.Nandakumar HOD** Department of cardiology, Kilpauk Medical College and Hospital, **Prof.G.Palanisamy, M.D., D.M., HOD** Department of cardiology, Royapettah Hospital for their valuable help, suggestion and granting me permission to carry out my project in their department.

I express my sincere thanks to **Prof.V.Meera M.D.,** for her valuable help and suggestion throughout my study.I express my thanks to **Former Assistant Professor Dr.M.VijayaLakshmi M.D., and Assistant Professors Dr.K.Rekha M.D.,Dr.K.Geetha M.D., Dr.G.Komala., M.D,Dr. A. Mariappan M.D.,**for their valuable help and suggestions throughoutmy study.

I am very much thankful to **Dr. S.Y.Jaganathan,** Assistant Professor, Department of Pathology, kilpauk Medical College, Chennai for guiding me in the biostatistics. I also thank Tutor **Dr.J.Arul moorthy** for his help in this study. The author expresses her special thanks to her colleagues for their immense help, constant encouragement and unconditional support throughout the study. The author is indebted to the patients and the persons from whom blood samples were collected for conducting the study.

Finally, the author expresses her special thanks to her husband Mr. Saravanan Muthaiah and to all her family members for the moral support and encouragement extended throughout the study by them.

**INSTITUTIONAL ETHICAL COMMITTEE**
**GOVT.KILPAUK MEDICAL COLLEGE,**
**CHENNAI-10**
**Ref.No.3393/ME-1/Ethics/2013 Dt:27.09.2013**
**CERTIFICATE OF APPROVAL**

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study on APOA1 Gene Polymorphism G-75A (RS1799837) & C+83T (RS5069) and its association with coronary artery disease" – For Project Work Submitted by Dr.G.Udayakumari, MD (Bio-Chem), KMC, Chennai-10.

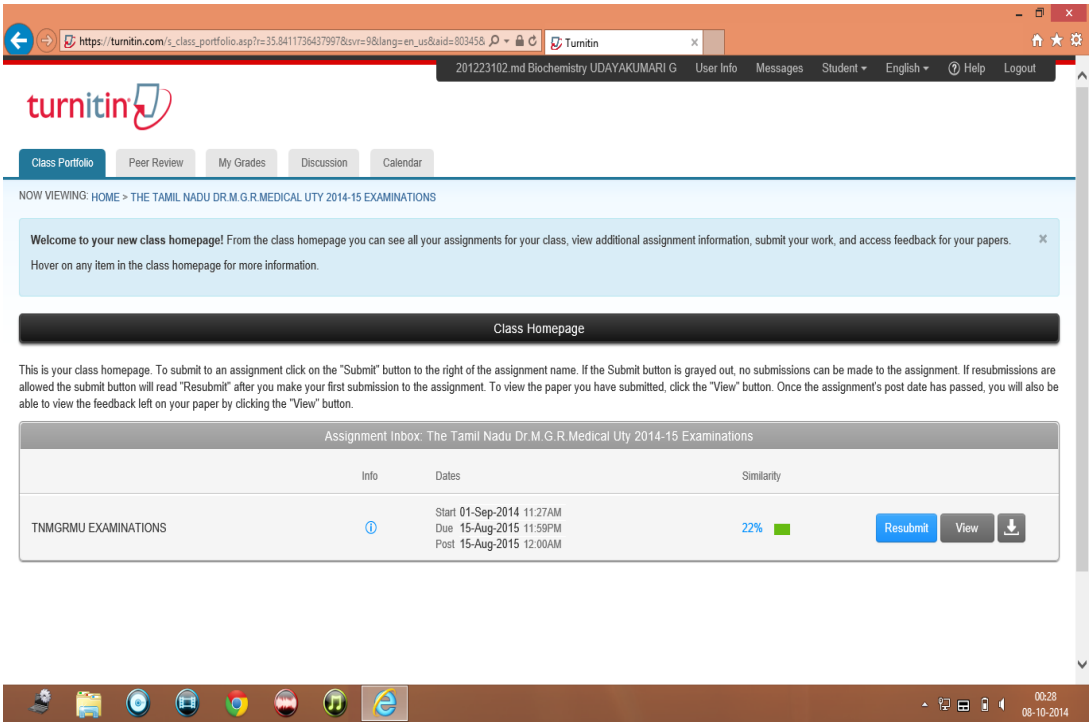
The Proposal is APPROVED.

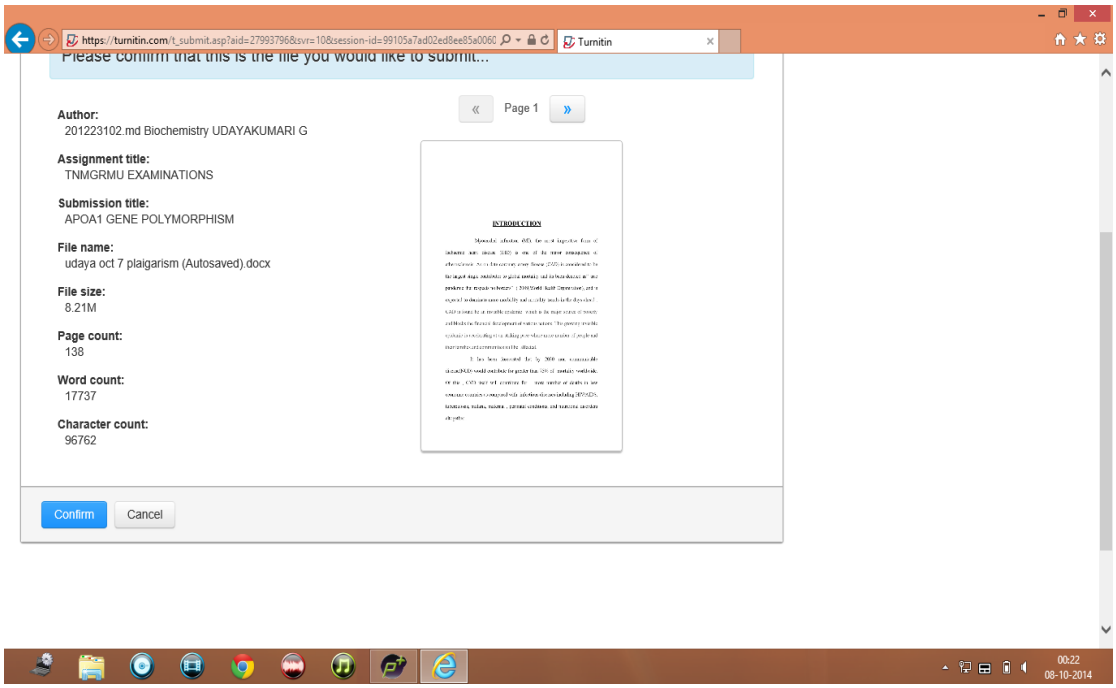
The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

25/11/13.

CHAIRMAN,
Ethical Committee
Govt.Kilpauk Medical College,
Chennai

GOVT. KILPAUK MEDICAL COLLEGE
25 NOV 2013
Kilpauk, Chennai-10.


https://turnitin.com/s_class_portfolio.asp?r=35.84117364379978svr=9&lang=en_us&aid=80345&
Turnitin
201223102.md Biochemistry UDAYAKUMARI G
User Info
Messages
Student
English
Help
Logout
turnitin
Class Portfolio
Peer Review
My Grades
Discussion
Calendar
NOW VIEWING: HOME > THE TAMIL NADU DR.M.G.R.MEDICAL UTY 2014-15 EXAMINATIONS
Welcome to your new class homepage! From the class homepage you can see all your assignments for your class, view additional assignment information, submit your work, and access feedback for your papers.
Hover on any item in the class homepage for more information.
Class Homepage
This is your class homepage. To submit to an assignment click on the "Submit" button to the right of the assignment name. If the Submit button is grayed out, no submissions can be made to the assignment. If resubmissions are allowed the submit button will read "Resubmit" after you make your first submission to the assignment. To view the paper you have submitted, click the "View" button. Once the assignment's post date has passed, you will also be able to view the feedback left on your paper by clicking the "View" button.
Assignment Inbox: The Tamil Nadu Dr.M.G.R.Medical Uty 2014-15 Examinations
Info
Dates
Similarity
TNMGRMU EXAMINATIONS
Start 01-Sep-2014 11:27AM
Due 15-Aug-2015 11:59PM
Post 15-Aug-2015 12:00AM
22%
Resubmit
View
00:28
08-10-2014


https://turnitin.com/t_submit.asp?aid=27993796&svr=10&session-id=99105a7ad02ed8ee85a0060
Turnitin
Please confirm that this is the file you would like to submit...
Author:
201223102.md Biochemistry UDAYAKUMARI G
Assignment title:
TNMGRMU EXAMINATIONS
Submission title:
APOA1 GENE POLYMORPHISM
File name:
udaya oct 7 plaigarism (Autosaved).docx
File size:
8.21M
Page count:
138
Word count:
17737
Character count:
96762
« Page 1 »
INTRODUCTION
Confirm
Cancel
00:22
08-10-2014

# ABBREVIATIONS

| | | |
|---|---|---|
| CAD | - | Coronary Arterial Diseases |
| CHD | - | Coronary Heart Disease |
| IHD | - | Ischemic Heart Disease |
| CVD | - | Cardio Vascular Disease |
| ESC | - | European Society of Cardiology |
| ACC | - | American Society of Cardiology |
| MI | - | Myocardial Infarction |
| STEMI | - | ST segment elevated MI |
| NSEMI | - | Non ST segment elevated MI |
| EDTA | - | Ethylene Diamine Tetra Acetic Acid |
| PCR | - | Polymerase Chain Reaction |
| SNP | - | Single Nucleotide Polymorphism |
| WHO | - | World Health Organisation |
| NCD | - | Non Communicable Diseases |
| DNA | - | Deoxyribonucleic Acid |
| RNA | - | Ribonucleic Acid |
| HGNC | - | HUGO Gene Nomenclature Committee |

| | | |
|---|---|---|
| TEA | - | Tris EDTA Acetate |
| HDL | - | High Density Lipoprotein |
| LDL | - | Low Density Lipoprotein |
| VLDL | - | Very Low Density Cholesterol |
| RCT | - | Reverse Cholesterol Transport |
| LCAT | - | Lecithin Cholesterol AcylTransferase |
| CETP | - | Cholesterol Ester Transfer Protein |
| ABCA1 | - | ATP Binding Cassette Protein A1 |
| SR B1 | - | Scavenger Receptor-B1 |
| VSMC | - | Vascular Smooth Muscle Cell |
| NF | - | Nuclear Factor |
| TF | - | Transcription Factor |
| HNF | - | Hepatocyte Necrosis Factor |
| PPAR | - | Peroxisome Proliferator Activated Receptor |
| DM | - | Diabetes Mellitus |

# CONTENTS

# ABSTRACT

## TITLE

**APOA1 GENE POLYMORPHISM G–75A (rs 1799837) AND C+83T (rs 5069) AND ITS ASSOCIATION WITH CORONARY ARTERY DISEASE**

Apo A1 shows a main part in preventing the evolution of atherogenesis which is the main causative event in Myocardial infarction(MI).A large number of genetic studies were conducted to find the association of various Apo A1 gene polymorphisms and susceptibility of MI in different populations. With this background, the candidate gene of this study is Apo A1gene at two Msp 1 restriction sites **G–75A transition in the promoter region and C+83T transition in the first intron.**

## AIM OF THE STUDY

Aim of the study were,

1. Is there any genetic polymorphism in Apo A1 gene at two Msp 1 restriction sites **G–75A transition in the promoter region and C+83T transition in the first intron** in the studied population?
2. To study the distribution of the Apo A1 polymorphic allele
3. To find the association between the above mentioned two Msp 1 restriction sites with respect to MI & Plasma lipid profile.

## MATERIALS AND METHODS

The study group included 52 patients who had documented MI and the control group included 52 age, gender and risk factor matched groups. Fasting venous blood was collected from each subject and estimations of Glucose, Urea, Creatinine ,Total cholesterol(TC) ,Triglyceride(TGL), HDL,LDL, Apo A1, Apo B estimations. Blood collected in EDTA coated tube was used for polymorphic studies.

## RESULT

Fasting serum levels of TC,TGL,LDL ,HDL, Apo A1and Apo B and Apo B/ Apo A1 ratio were estimated . TC,TGL,LDL level were significantly higher in cases compared with controls. Serum HDL, apo A1 level was significantly lower in the cases than in the control group and that the serum apo B level and apoB/apoA1ratio in the cases was significantly higher than in the control group. The lipid variables compared across genotypes at two polymorphic site is as follows

## POLYMORPHIC STUDIES:

**I.** The C+83T (FIRST INTRON) polymorphism reveals that 'TT' homozygous genotype was higher among cases and 'CC' genotype was seen more in controls, CT more or less equal among cases and controls. Genotype difference between cases and controls were statistically significant (*p* - 0.006). As far as frequency, 'T' allele was higher among cases (0.36) as compared to controls (0.13); and frequency of 'C' allele was higher among

controls (0.86) as compared to cases (0.63). No statistically significant differences were observed between C allele and T allele carriers for any lipid variables other than HDL.

**II.** For G-75A (PROMOTER SITE) polymorphism, genotypes across the cases and control reveals that 'GG' homozygous genotype was higher among cases and 'GA' genotype was seen more in controls, AA more or less equal among cases and controls. But the difference was statistically insignificant. As far as frequency, 'G' allele was higher among cases (0.75) as compared to controls (0.69); and frequency of 'A' allele was higher among controls (0.30) as compared to cases (0.25).In GA genotype mean HDL,Apo A1 were high and low Apo B/Apo A1 ratio as compared to GG genotype. But no statistically significant differences were observed between G allele and A allele carriers for any lipid variables

## CONCLUSION

- In this study T allele is significantly increased in cases compared to controls which implies that the presence of T allele present in C+83T (first intron) of the Apo A1 gene, might increase the development of MI. When compared across the lipid variables , no significant difference were obtained other than HDL, since various factors influences the Apo A1 gene expression.

- In this study, no statistically significant differences were obtained across genotype as well as lipid variables in G-75A (promoter) region of Apo A1 gene for the development of MI.

**KEY WORDS**

Myocardial infarction ,Apo A1, Apo B, HDL,LDL, Polymorphism ,Genotype , C+83T(first intron),G-75A(Promoter site), Lipid Variables.

# INTRODUCTION

Myocardial infarction(MI), the most imperative form of Ischaemic heart disease(IHD) is one of the major consequence of atherosclerosis. As on date coronary artery disease (CAD) is considered to be the largest single contributor to global mortality and it's been denoted as"true pandemic that respects no borders" (2009,World Health Organization) and is expected to dominate more morbidity and mortality trends in the days ahead .CAD is found be an invisible epidemic which is the major source of poverty and blocks the financial development of various nations. This growing invisible epidemic is accelerating at a striking pace where more number of people and their families and communities will be affected.

It has been forecasted that by 2030 non communicable disease(NCD) would contribute for greater than 75% of mortality worldwide. Of this, CVD itself will contribute for more number of deaths in low economic countries as compared with infectious diseases including HIV/AIDS, tuberculosis, malaria, maternal , perinatal conditions, and nutritional disorders altogether .

As the Human Genome Project study was completed ,this in turn helps us to identify the genetic, physical and Single nucleotide polymorphism(SNP) maps of the human genome and this provides the opportunity to map and identify the susceptibility genes for not only single-gene (Mendelian) disorders , but also complex polygenic (non-Mendelian) traits. The evidence is well distinguished for heritability of MI(the consequence of CAD), so it is well established that the inheritance being one of the most important risk factor for this polygenic trait.

The saying "complex trait" as such indicates interaction between genes and with environment , along with the complex inheritance which is common and highly probabilistic in phenotypic manifestation as compared to "simple" Mendelian traits. The impact of genetic factors are more important, and recent research in this field has led to identifying candidate genes associated with elevated risk of susceptibility for MI.

The Current study in genetic cause for MI is being unravelled at an accelerated pace. The future assessment of a person's lifetime risk for developing atherosclerotic vascular disease by genetic analysis ,formerly an idea is now evolving into reality. These findings could help

in lifestyle modification and the choice and dosage of specific drugs. The best way to fight the burden of CAD is the preventive approach.

The trends in CAD mortality has been projected to shift from infectious to NCD over the next few years .The most affected population would be the poorest in low & middle-income countries and this economic groups are going to be disproportionally affected , with incidence being equal in both men and women . At the household level, there is an ample evidence to ascertain that CAD and other NCD contribute to poverty due to tragic illness like MI leading to high spending either through out of pocket expenditure or health policies spending by the government agency.

At macro-economic level, CAD is found to place a huge affliction on the financial prudence of low and middle economic countries.NCD including CAD and diabetes mellitus (DM) are estimated to reduce the Gross Domestic Product in low and middle economic countries which results in low financial growth, as many people are expected to die below the average span of life.

People in low and middle economic nations die prematurely from CAD and other NCDs since ：

1. Since these people have less access to affordable and proper medical facilities including early detection of diseases.

2. More exposed to factors that contribute to risk events like MI, such as alcohol, tobacco.

3. They predominantly do not have the benefit of access to various prevention programmes as compared with high economic group people.

Asian Indians--living both in India and abroad is found to have highest rates of CAD in the world. The CAD among Indians is usually more aggressive at the time of presentation compared with any other population . The overall impact is considered to be much higher because the CAD in Asian Indians affects the "younger" working class population which will affect dizzying economic boom ,for a developing country like India and to sustain this growth it should have a healthy populace.

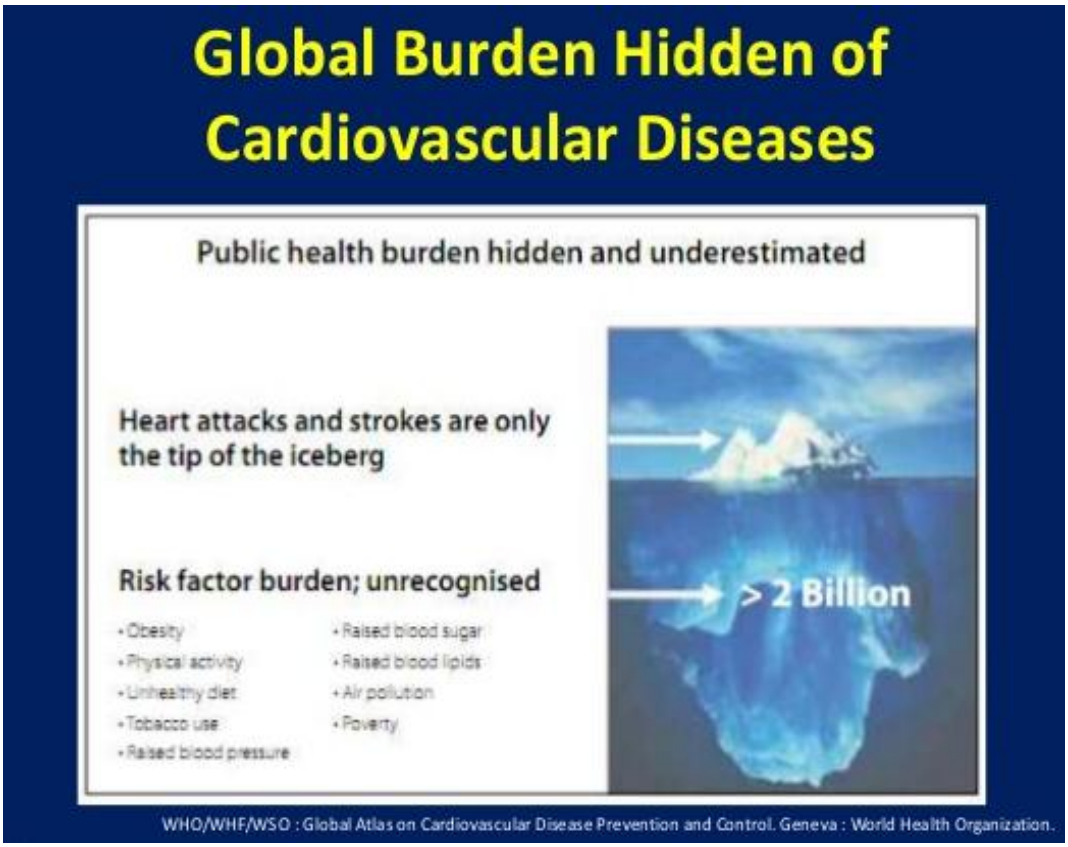
The effect of established, as well as novel, risk factors is multiplicative in an exponential manner. Occurrence of family history

has been established in NCD like Hypertension (HT), DM, and CAD suggests that genetic basis has to be explored .

Various traditional, non-traditional and novel risk factors are involved in the development of coronary artery disease which on in due course led to the development of dreadful complication, MI. The above mentioned risk factors has been ineffectual in completely predicting the progression of atherosclerotic changes , suggesting that specific genetic predisposition should be taken under consideration.

Lipoproteins are involved in the pathogenesis of atherosclerosis. Epidemiologic studies have established that , there exist an transposed relationship between serum levels of high-density lipoprotein (HDL), apo A1[11.13], the main component of HDL and the occurrence of CAD (antiatherogenesis), whereas low density lipoprotein (LDL) has been established as an proatherogenic factor.[8-10,52] The competency of reverse cholesterol transport (RCT) depends on the functional ability of apo A1 and to form mature HDL [14,15] by its capacity to promote cellular cholesterol efflux, lipid binding function and activation of an enzyme lecithin cholesterol acyltransferase (LCAT).Also efficacy depends on interaction with precise receptors and lipid transfer proteins. In assessing the risk for CAD there is an ample evidence to suggest that

**FIG:1**


Global Burden Hidden of Cardiovascular Diseases
Public health burden hidden and underestimated
Heart attacks and strokes are only the tip of the iceberg
Risk factor burden; unrecognised
• Obesity
• Physical activity
• Unhealthy diet
• Tobacco use
• Raised blood pressure
• Raised blood sugar
• Raised blood lipids
• Air pollution
• Poverty
> 2 Billion
WHO/WHF/WSO : Global Atlas on Cardiovascular Disease Prevention and Control. Geneva : World Health Organization.

apo A1 particularly, ratio between apo B/apo A1 are strong predictors than HDL/LDL ratio[9-11].

There are several investigations that are useful after the disease has set in. Genetic analytic study will be the screening tool before the development of CAD. In future the genetic analysis may lead to the development of Gene therapy mechanism which could be useful in the treatment of CAD and for the prevention of MI and will provide more insight on the genetic basis of CAD, to the circumstances leading to the infarct.

Various studies established relationship between genetic polymorphism in ApoAI-CIII-AIV gene cluster and its association with variation in the levels of Triglycerides, HDL , Apo A1 levels , premature atherosclerosis, MI and CHD in different populations .[2,3-7]The ApoAI-CIII-AIV gene cluster approximately fifteen kb size and is found on chromosome 11q23.3[1]This Closely placed gene complex which evolved from the same evolutionary sequence are thought to have significant role in lipid and lipoprotein metabolism.[2] Genetic variation in this gene cluster will affect the gene expression in the hepatocyte as well as in the intestinal epithelial cells.

The purpose of my study is to explicate the relationship between two SNPs in the Apo A1,11q 23.3, namely the G-75A (Promoter region) and C+83T (First intron)with Myocardial infarction which is the sequel of CAD

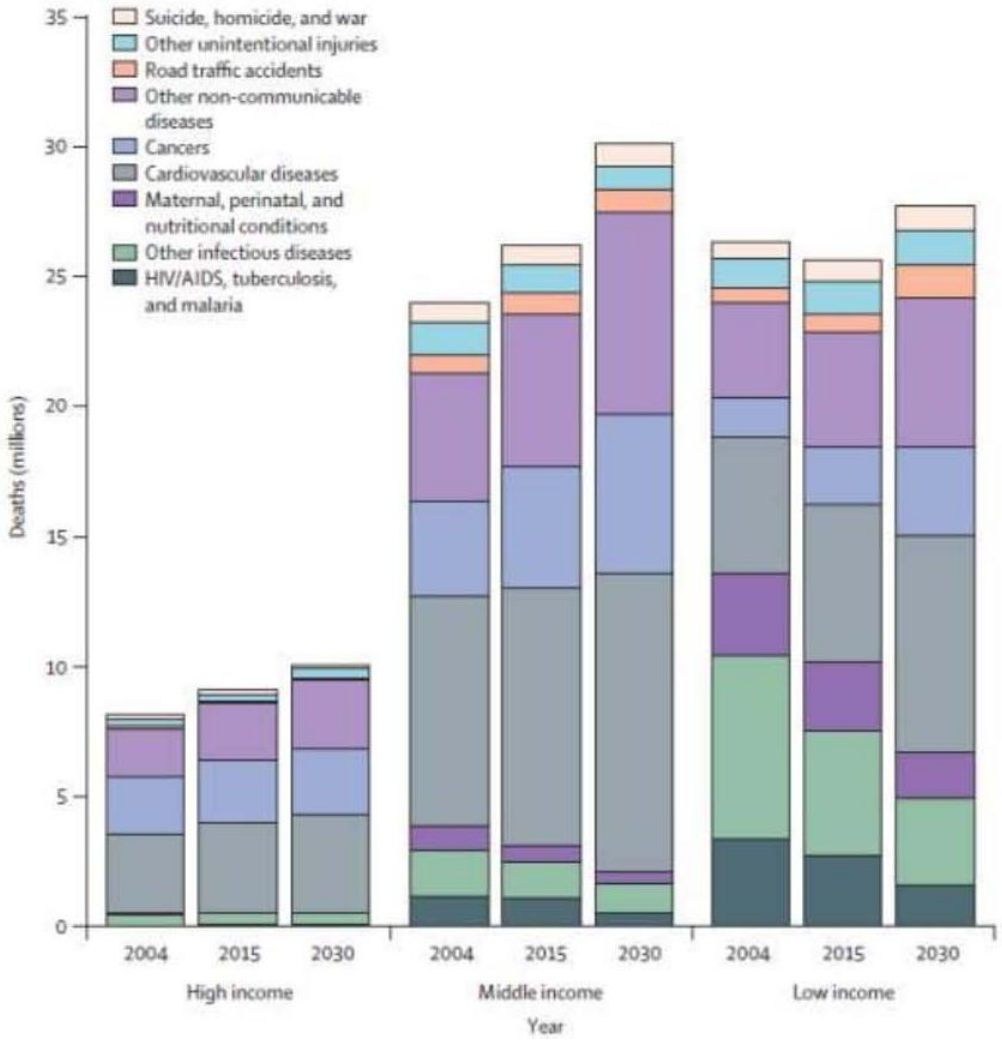
## REVIEW OF LITERATURE

MI results in group of closely related syndromes generically designated as Ischaemic Heart Disease (IHD). Atherosclerotic coronary arterial obstruction contributes more than 90% of MI. Hence it is termed as CAD or CHD. Onset of coronary atherosclerosis begins during childhood/adolescence and IHD is the late manifestation of this progressive events.

### CLINICAL MANIFESTATION OF IHD[84]:

- MI
- Angina Pectoris
- Chronic IHD with heart failure
- Sudden cardiac death

**FIG : 2 HISTOGRAM REPRESENTS THE PROJECTED TRENDS&EXPECTED SHIFTS OF COMMUNICABLE TO NCD ACROSS LOW,MIDDLE AND HIGH INCOME GROUPS OVER THE NEXT FEW DECADES**.



## EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE(CVD)

According to global burden of disease survey (GBD)[34] the estimated mortality in India with CHD was 1.6 million in the year 2000.With the same trend being continued its estimated that in 2008 ,17.3 million people died due to CVD, which accounts for 30 percent of all global deaths. Out of the total mortality reported 7.3 million were due to CHD and 6.2 million due to stroke. It is predicted by the year 2015, approximately 64 million cases of CVD are likely, of which nearly CHD cases accounts for 61 million. (the remaining would consists of stroke, rheumatic heart disease and congenital heart diseases). Mortality from this cluster of diseases are anticipated to hit at a staggering rate of 3.4 million. Its alarming that by the time 2030 total number of people who will die from CVDs, which constitutes mainly heart disease and stroke, will increase to reach 23.3 million[21]

There is a substantial unevenness in the shape and magnitude subsequently from 1950s with respect to mortality drift of CHD across various countries in the globe. Even among nations within the same topographical region, CHD mortality trends are not consistent.

There were three CHD trending mortality patterns were perceived :

1) Rise-and-Fall pattern[26]-prominent in high economic growth countries (including Anglo-Celtic, Nordic and North western Continental European countries as well as in the United States of America and Australia). Here the mortality rates are elevated, peaked (in 1960s or early 1970s) and have fallen swiftly , by an average of about 50 percent.

2) Rising pattern : CHD mortality rates have steadily increased which indicates an ongoing epidemic ;this type is distinguished in Eastern European and former Soviet countries, south east Asia where mortality trends is found to drastically increase at an alarming speed and in these countries the highest mortality rates were recorded.

3) Flat pattern[26] : Notable in Japan and several European Mediterranean countries where CHD mortality rates have persisted relatively low and stable following the flat pattern (Beaglehole,1999; Mirzaei et al., 2009)[94].

In identifying the genomic basis of complex traits like CAD we are still in the early phase .From the panoramic angle of public health problem, no other polygenic trait is more vital than atherosclerotic CAD

and MI and it would be expected to be the number one cause of mortality worldwide by 2020. Linkage analysis, Genome wide association studies (GWAS)[33] and specific genetic epidemiologic studies have, in cumulative , initiated to open up this field and it provide more understanding to the candidate genes underlying this common and most important condition.

## MYOCARDIAL INFARCTION

## EVOLUTION OF DEFINITION

The World Health Organization (WHO) defined MI from clinical symptoms, abnormalities traced in ECG and serum/plasma enzymes levels. Now a days ,with the greater advancement in the availability of precise imaging techniques and more advancement in the clinical biochemistry which enables in detecting the cardiac marker enzymes, even when a tiny focus of myocardial necrosis is present. Therefore, present clinical practices, health care delivery models, epidemiology and clinical trials would require a more specific definition and re-assessment of previous definitions of MI.

Accuracy of detecting MI has been evolved over the years .Such evolution were occurred when glutamine oxaloacetic transaminase (GOT) was substituted with lactate dehydrogenase (LDH)[4] and

advanced by creatinine kinase (CK) and the MB fraction of CK, i.e. CKMB activity and CKMB mass. The major issues lies in the identification of MI, hence the European Society of Cardiology (ESC) along with American College of Cardiology(ACC)organised a consensus conference in 1999 to revise the definition of MI (published in the year 2000 ).

Update of 2000 consensus document was made under the headship of ESC, ACC, and American Heart Association (AHA) was summoned, together with World Heart Federation (WHF), a global Task Force committee, due to the substantial advancement in the diagnosis and treatment of MI over the years from the initial document was made. From various perspectives the Global Task Force embraces various number of working groups in order to enhance the ESC/ACC criteria for the diagnosis of myocardial infarction to update the previous established criteria.

The Task Force identifies that this explanation for the definition of MI will be exposed to diverse changes in the forthcoming days, as a result of scientific expansion. Therefore, its clearly understood that the definition is still not the final version for all time. The present definition will further undergo refinement in the expected days to come.

## CRITERIA FOR DETECTING ACUTE MYOCARDIAL INFARCTION[16,17,18]

The Acute MI is termed when there is evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischemia.

The following are the criteria meets the diagnosis for MI:[66]

Rise or fall of cardiac biomarker values (preferably cardiac troponin (cTn)] with at least one value above the 99th percentile upper reference limit (URL) and with at least one of the following:

- Ischemia symptoms
- Pathological Q waves traced in Electrocardiography.
- New or recognised significant ST-segment–T wave (ST–T) changes
- Occurrence of New left bundle branch block (LBBB).
- ECHO findings of new loss/ regional wall motion abnormality of viable myocardium
- Angiography or Autopsy findings of intracoronary thrombus

**WORLD WIDE - CLINICAL CLASSIFICATION OF DIFFERENT TYPES OF MI**[16,17,18]

Type One : Spontaneous MI

Type Two: MI secondary to an ischemic imbalance

Type Three: MI resulting in death .

Type Four a: MI related to per cutaneous coronary intervention (PCI)) imaging.

Type Four b: MI related to thrombosis due to stent procedure.

Type Five : MI related to coronary artery bypass grafting (CABG)

**PATHOLOGICAL DEFINITION:** pathologically it can be defined as

1. **ACUTE** - Acute MI is regarded by the recruitment of neutrophils at the affected focus. If the time interim is quite narrow e.g. six hours between the beginning of the infarction and death, minimal or no neutrophils may be seen in the affected focus[4].

**2. HEALING**–When the neutrophils are replaced by the recruitment of mononuclear cells and fibroblasts it characterises the MI at the healing stage.

**3. HEALED** –Healed stage is represented by the presence of Scar tissue without cellular intrusion. The complete process leading to the

formation of scar tissue usually lasts for approximately five to six weeks[4] . Reperfusion alters the microscopic and macroscopic features of the cardiac myocyte with the appearance of contraction band necrosis and large quantities of extravasated erythrocytes.

## CLASSIFICATION OF MI BASED ON ELECTROCARDIOGRAPHIC FINDINGS[5]

- ST elevation (STEMI) MI
- NON STEMI

Management practice guidelines helps in discerning STEMI and non-STEMI, as most of the epidemiological studies on which endorsements were made, but it does not discriminate transmural from nontransmural MI.

## CLINICAL FEATURES OF MI:

Myocardial ischemia precedes the development of MI .

## TYPICAL SYMPTOMS[18,27]:

- Various mixtures of discomfort in chest, epigastric region, upper extremity, mandibular area (with exertion or even at rest)
- Ischemic corresponding including fatiguability or dyspnea .
- Discomfort duration associated with AMI usually lasts for more than twenty minutes.
- Diffuse discomfort- which is not positional/not localised /not affected by movement of the region.
- Convoyed with diaphoresis, nausea or syncope.
- Above mentioned symptoms are not precise for MI, this could be misdiagnosed and attributed to gastrointestinal, pulmonary ,neurological or musculoskeletal disorders.

## OCCURANCE OF MI WITH ATYPICAL SYMPTOMS[27]

- Palpitations or Cardiac arrest
- MI deprived of symptoms can occur in DM , Women, Elderly, Post-operative and critically ill patients.

  Atypical symptoms can occur when there is a increasing or decreasing pattern of cardiac biomarkers, so cautious assessment of these patients is advised.

## RISK FACTORS OF MI:[19,20,21]

INTERHEART STUDY CONDUCTED ACROSS 52 COUNTRIES IN SOUTH ASIAN COMPONENT WITH THE KEY RESULT OF

Nine risk factors that contribute to ninety per cent of the population attributable risk (PAR) in men folk and ninety four per cent in womanhood category.

- Compared to western population, deaths from AMI in south Asians were five to ten years earlier.
- South Asian men faces AMI 5.6 years younger than women.
- Nine conventional risk factors[30]are lipid profile abnormality, smoking, HT, DM, obesity particularly abdominal, psychosocial factors, amount of ingesting fruits & vegetables, liquor consumption and regular physical activity altogether explains eighty six percent of the AMI risk in south Asians.
- **The most important risk factor in south Asians are atypically high ratio of Apo-B/ApoA-1 (higher in south Asians)[22]and smoking[30]cadre.**

- Low literacy rate is associated with augmented risk of AMI worldwide.

- South Asians have low protective lifestyle factors such as high vacation time , physical exertion and balanced ingestion of dietary fruits and vegetables than western population,

- Significantly increased waist-hip ratio in south Asians.

- Regular liquor consumption is not protective for AMI in south Asians.

However occurrence of MI is pertained to higher age groups, the actual incidence depends on predisposing risk factors for atherosclerosis. Any risk factor when it is associated, actually doubles the relative risk of developing atherosclerotic CAD.

**Hyperlipidemia:**

Increased risk of coronary atherosclerosis and MI is associated with the rise in the levels of total cholesterol, LDL, triglycerides or lower HDL level< 40 mg per decilitre.

## Diabetes Mellitus

DM patients either insulin-dependent or non–insulin-dependent , they are at greater risk of development and progression of CVD [29].DM not only increases the rate of evolution of atherosclerosis changes in the coronary arteries but it also affects the variability in lipid profile.

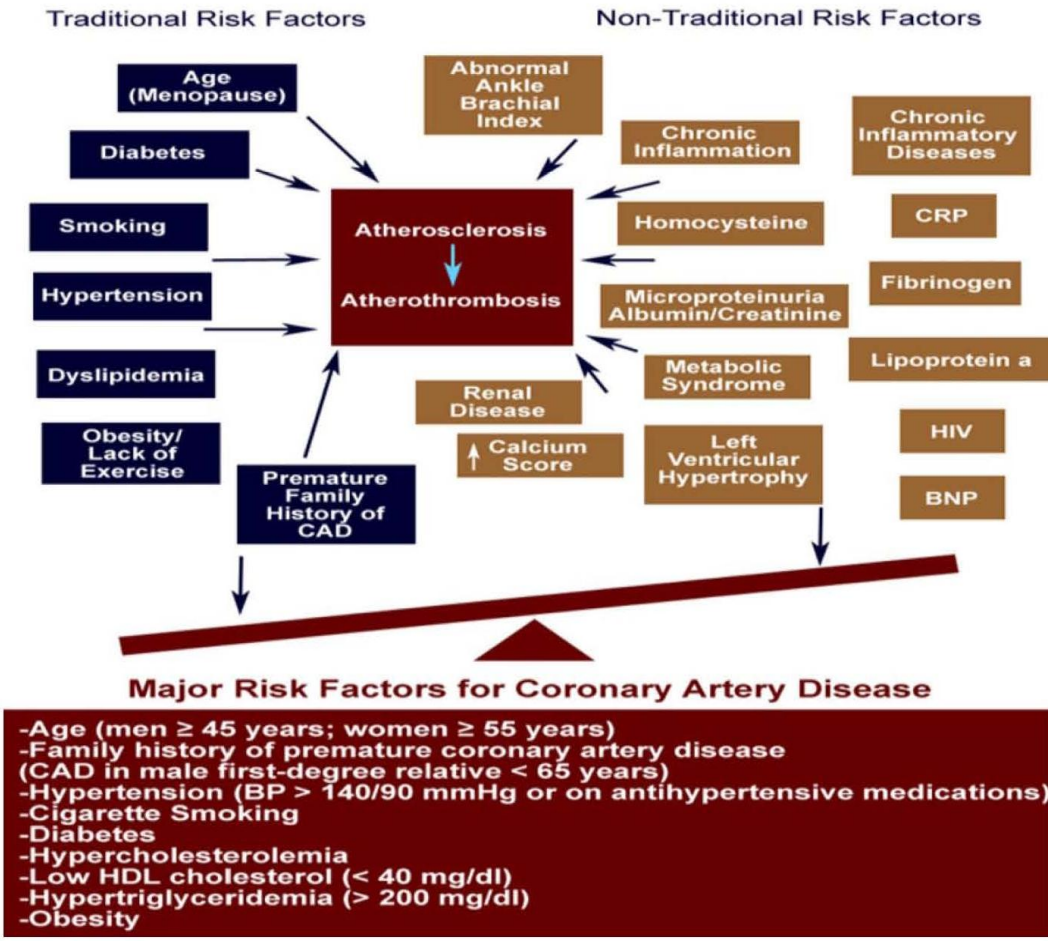
## Hypertension

Either systolic or diastolic HT, is consistently associated with an augmented risk of occurrence of MI. This risk factor is significantly reduced with the intake of appropriate drugs for elevated blood pressure.

## Tobacco Use

Blood vessel damage can occur with the consumption of certain components of tobacco and tobacco combustion gases[58]. Atherosclerotic formation and progression occurs due to the body's response to this type of injury, thereby increasing the risk of MI. Smoking acutely increases thrombus formation by platelets.

**FIG : 3 MAJOR RISK FACTORS OF CAD**



**Male Gender:**

Atherosclerotic vascular disease and MI incidence is higher in menfolk than women at all age groups[5]. With increasing age this gender difference in MI, however, narrows.

**Family History**

The source of familial coronary events is multifactorial and encompasses components, such as genetic mutations/polymorphisms and its interaction with various environmental factors (e.g. smoking, high-fat diet, exercise). Therefore positive family history of premature coronary disease increases one's risk of developing atherosclerosis and MI.

**PROOF OF ASSOCIATION BETWEEN PREDICTIVE VALUE OF APO A-I , B AND CAD BY FOUR RECENT PROSPECTIVE STUDIES[83]**

1. The Quebec Cardiovascular Study - this was the first prospective study to show that elevated serum levels of apo B remained as an self determining risk factor for predicting ischemic coronary events and it has been determined that this association is higher in men with appropriate levels of LDLc.

2. AMORIS[11] (Apolipoprotein-related Mortality Risk Study) - showed the apo B/apo A-I ratio was the distinct variable that was more strongly associated with higher risk of developing fatal MI, as compared with other lipid ratio's .

3. INTERHEART[84]study –when compared with several conventional risk factors like smoking, HT, DM, stress, obesity particularly abdominal and regardless of other variables like gender, age and ethnicity, the ratio of apo B/apo A-I was more sturdily associated with predicting MI .

4. MONICA/KORAstudy - The main findings of this study was the strong direct association between serum levels of apo B and risk of developing MI, whereas reciprocal relation exists with apo A-I levels and development of MI. However, even after adjustments to age, body mass index, smoking, DM and HT, the multivariate analysis showed ratio of apo B and apo A1was strongly allied with the risk of MI.

Prediction of cardiac risk is attributed to increased apo B levels in some studies and in other studies it has been due to diminished Apo AI levels. But according to literature its evident that the balance between

atherogenic and antiatherogenic factors which is echoed by the ratio of apo B/apo A-1, represents an additional and important risk prediction parameters for CVD, and of now it is considered a better indicator when compared to lipids, lipoproteins and traditional lipid ratios.

## PATHOGENESIS

When there is diminished blood supply to the heart, myocardial ischemia results [94], if it exceeds a critical threshold and the capacity to maintain the normal function and homeostasis with the defect in myocardial cellular repair mechanism[18], MI occurs. Ischemia at this acute threshold level for a prolonged duration, results in irrevocable myocardial cellular damage or necrosis. Increased myocardial metabolic demand, reduced provision of oxygen and nutrients to the myocardium through the coronary artery circulation, or both contributes critical myocardial ischemia. Superimposed thrombus on an ulcerated or unstable atherosclerotic plaque, it occludes the coronary circulation which then interrupts the supply of myocardial oxygen and nutrients. MI can also be precipitated when more than seventy five percent fixed coronary artery stenosis associated with coronary vasospasm caused by atherosclerosis or a dynamic stenosis also limits the supply of oxygen and nutrients.

Myocardial metabolic demand is increased in conditions like dissipation of physical exertion, HT (severe type- including forms of hypertrophic obstructive cardiomyopathy)and extreme stenosis of aortic valve. In addition to that, other cardiac valve abnormalities and reduced cardiac output states also causes decreased mean aortic pressure, which precipitates MI.

Disruption in the vascular endothelium is the cause for most of the MI events. This disruption which ultimately results in unstable atherosclerotic plaque formation , that culminates in intracoronary thrombus leading to blockage in blood flow of coronary arteries. If such an blood flow block continues for more than twenty minutes, irrevocable myocardial cell damage and necrosis will occur.

Over a period of years to decades atheroma initially starts as a fatty streak , then progress to full fledged atherosclerotic plaque. Lipid-rich core covered over by fibromuscular cap are the two key characteristic features of the clinically expressed atherosclerotic plaque. The area where the structural stability of an atheromatous plaque is often lost is at the shoulder region (juncture of the fibromuscular cap and the vessel wall). Fibromuscular cap is weakened by the activity of matrix metalloproteases and the release of other collagenases and

proteases in the plaque, which results in plaque rupture. Endothelial disruption and fissuring or erosion of the fibromuscular cap can also due to hemodynamic forces applied to the arterial segment in addition to action of proteases.

The myocardial necrosis originally starts in the endocardial area more distal to the coronary arterial supply. As the time duration is prolonged, the coagulative necrosis also enlarges, extending from the endocardium to the myocardium and eventually reaches the epicardium. The necrosis area then spreads laterally to watershed areas or collateral perfusion. After six to eight hour period of coronary block, most of the distal myocardium get necrosed. The extent of necrosis defines the magnitude of the MI. More striated cardiac muscle can be saved from irrevocable damage or death if the blood flow is re-established.

The three key determining factors of MI are : 1) Level of the block in the coronary artery 2) length of the obstruction period and 3) Presence or absence of collateral circulation.

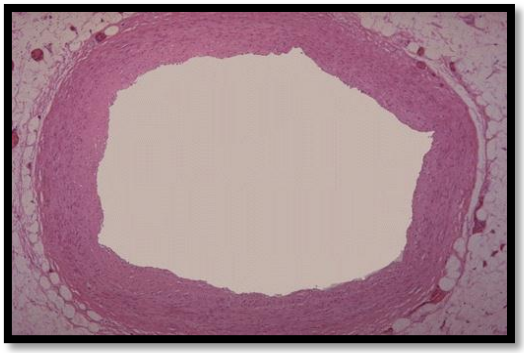
STEMI : Complete blockade of coronary artery after plaque rupture results in STEMI . Most often this is due to the plaque that previously caused less than fifty percent occlusion of the lumen.

NSTEMI : Usually allied with greater plaque burden without complete blockade. This difference enhances the increased early mortality seen in STEMI and the ensuing equalization of mortality between STEMI and NSTEMI after one year.

## ATHEROGENESIS

The normal arterial wall consists three well-defined layers[24,25]:

- Intima layer- is the subendothelium of vessel wall.
- Media – it includes vascular smooth muscle cells(VSMC)
- Adventitia- periphery of the vessel wall, composed of looser connective tissue



**NORMAL CORONARY ARTERY**

**ATHEROSCLEROTIC CORONARY ARTERY WITH THROMBOSIS**

## NORMAL ARTERIAL ENDOTHELIUM- BARRIER TO ATHEROSCLEROSIS:[24,25]

- Repels cells and inhibits blood clotting
- Surface which repels cells floating in plasma (including platelets)- strongly antithrombotic
- Substances can penetrate endothelium either through junctions between the endothelial cells, or by transgressing the cells themselves.
- An important function, controlled by the endothelium, is the ability to dilate (vasodilatation) and to constrict (vasoconstriction) and thus regulate tissue and organ blood flow

## KEY EARLY EVENT IN ATHEROSCLEROSIS :

- Is Damage to the endothelium
- main components are: 1. Endothelial dysfunction 2. Lipid deposition, and 3. Inflammatory reaction in the vascular wall these three eventually result, not only in the formation of atherosclerotic plaques, but in the entire arterial wall remodelling

This whole process in motion is maintained, by an intense cross-talk between endothelial cells, VSMC, and plasma-derived inflammatory cells, macrophages, and lymphocytes

The cross-talk involves an array of chemokines, cytokines, and growth factors which cause attraction of cells to the sites of atherosclerotic lesions, induce cell migration, proliferation, apoptosis, and the excess production of extracellular matrix

## 1. ENDOTHELIAL DAMAGE

Initially the damage is functional , the endothelium becomes more permeable to lipoproteins which move beneath the endothelial layer, in the underlying intima. Also the normal endothelium loses its cell-repellent quality allows inflammatory cells into the vascular wall. Later, the endothelium may become physically damaged, or even completely destroyed

## 2. LIPID ENTRY – KEY PROCESS IN ATHEROSCLEROSIS

Dysfunctional endothelial surface expresses ADHESION MOLECULES (Selectins)

Promotes adhesion of monocyte(precursor of macrophages)& Tcells via VCAM ('rolling' interaction of cells with endothelium )

Adhered cells stimulated by the monocyte chemoattractant protein-1 (MCP-1)

Crosses the endothelium and lodges in the intima

Monocytes transform into resident macrophages (By Monocyte colony-stimulating factor (M-CSF) secreted by endothelial cells& VSMC) and produce reactive oxygen species and at the same time T lymphocytes also enter the vascular wall at this stage.

LDL oxidation in the intima.

Increased expression of macrophage scavenger receptors (scavenger receptor A, and CD36)

Hormone angiotensin also causes increased expression of VCAM-1 and MCP-1 & Nitric oxide production decreases in the damaged endothelium.

## 3. FUNDAMENTAL ROLE PLAYED BY INFLAMMATION

- The exit of monocytes and T lymphocytes from lumen and their activation in the intima are parts of the inflammatory response.
- Possibly there is a molecular mimicry between the antigens involved in atherosclerosis and the exogenous pathogens
- Such putative antigen(s) might be infectious agents, or molecules modified by reactive oxygen species. For instance, phosphorylcholine group found in the oxidized LDL is also a component of the capsular polysaccharide of bacteria.

## BOTH INNATE AND ADAPTIVE IMMUNITY INVOLVEMENT IN ATHEROSCLEROSIS

- Innate immunity includes recognition by scavenger receptors A, CD36
- Molecules which possess patterns encoded in immune memory bind to these receptors
- They activate cells through, pathway involving the transcription factor NF-κB.
- T cells, involved in adaptive immunity, are present in both early and late atherosclerotic lesions.
- Finally, circulating IgG and IgM-type antibodies produced against modified LDL

## ANOTHER HALLMARK OF ATHEROSCLEROSIS

## HYPERCHOLESTEROLEMIA

- Contributes to the induction of VCAM-1 and MCP-1
- Smaller size lipoproteins, the remnants and the LDL, are the most atherogenic , partly since they enter the vascular wall more easily.
- After leaving plasma, the LDL are modified in a way which enhances their potential to cause damage.
- Enzymes such as lipoxygenases, myeloperoxidase, and NADPH oxidases present in the activated macrophages facilitate LDL oxidation
- Oxidized LDL expresses VCAM-1 and MCP-1.
- They are cytotoxic to the endothelial cells and they are mitogenic for macrophages.
- LDL 'driver' apolipoprotein, apoB100, once oxidized, binds to the scavenger receptors, but not subjected to regulation by the intracellular cholesterol level.
- Macrophages overloaded with oxidized LDL form foam cells.
- Conglomerates of these cells are visible in the arterial walls as yellow patches ( fatty streaks)

- Dying foam cells release lipid that pools within the intima.
- These lipid accumulation become centres of the atherosclerotic plaques

## STRUCTURAL CHANGES IN ARTERIAL WALL DUE TO MIGRATION & PROLIFERATION OF SMOOTH MUSCLE CELLS

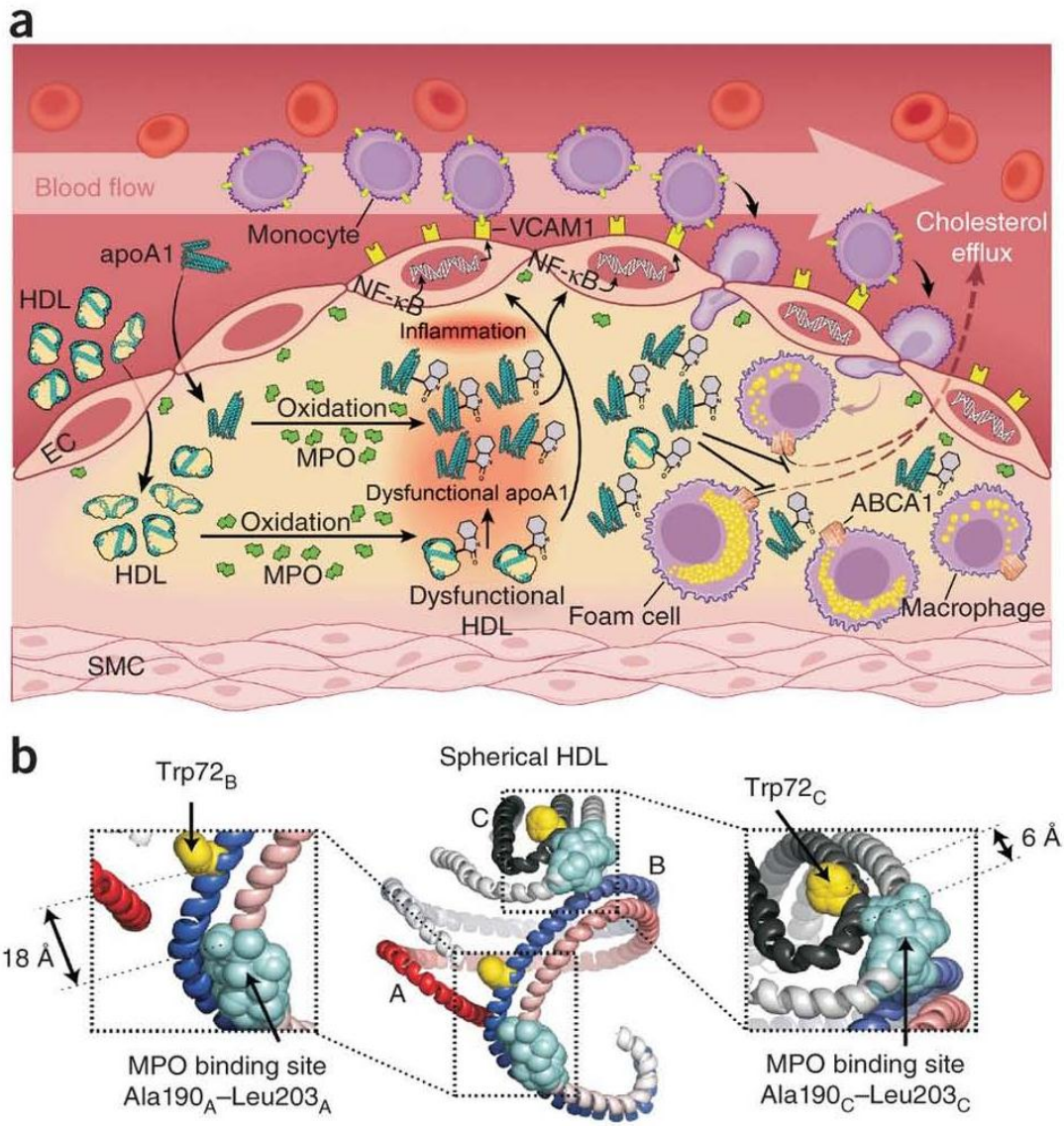
- All the above is accompanied by profound changes in the behaviour of VSMC.
- VSMC stimulated by growth factors such as the platelet-derived growth factor , the epidermal growth factor and the insulin-like growth factor-1.
- Migrate and proliferate toward the lumen of the arterial wall .

  They secrete
- Adhesion molecules and
- MCP-1

As do the endothelial cells, it also secretes variety of cytokines and growth factors like interleukin-1 (IL-1) and tumor necrosis factor (TNF-α).

- Importantly, activated VSMC also synthesize extracellular matrix, in particular collagen, which is deposited in the plaque.

**FIG : 4 FIGURE REPRESENTING MECHANISM OF ATHEROSCLEROSIS**



- As a result, the normally ordered structure of the arterial wall becomes completely disrupted, and the forming plaque may protrude into the lumen of the artery, interfering with the flow of blood.

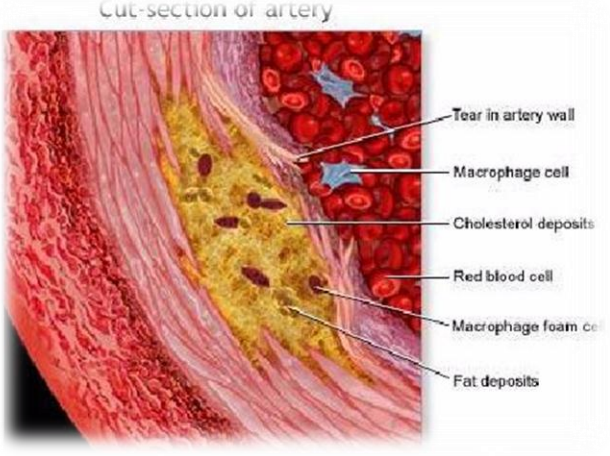
## ATHEROGENESIS

## COMPONENTS OF ATHEROMATOUS PLAQUE[37]

- Centre composed of - the foam cells and the lipid pool
- Periphery is fibrous 'cap' over the lipid pool- formed by collagenous matrix secreted by VSMC which had migrated into the intima and contains VSMC themselves, macrophages, and T lymphocytes.

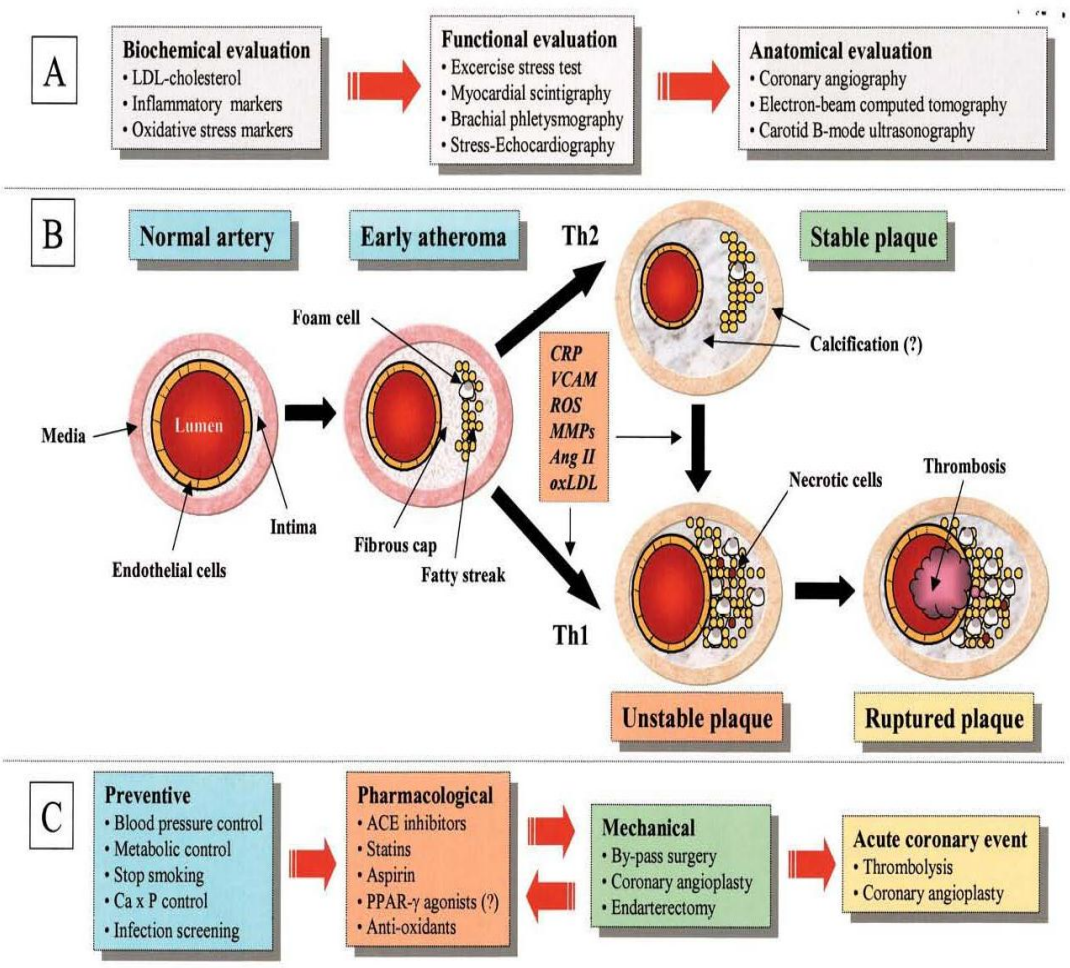
Mature atherosclerotic plaque-The lipid pool and fibrous cap penetrates the arterial wall on one hand and on the other side obstructs the arterial lumen CAUSING SEVERE MI

Parts of the more advanced lesions may also become calcified.




ATHEROSCLEROTIC PLAQUE GROW SLOWLY – SUDDEN RUPTURE IS THE REAL DANGER:

**FIG : 5 EVOLUTION OF ATHEROSCLEROSIS TILL ITS ACUTE RISK EVENTS**



## PATHOLOGICAL HALLMARKS OF MI

Most commonly documented in the infarct area supplied by the affected coronary arteries are

- Lipid-rich atheromatous plaque rupture,
- Haemorrhage inside the plaque and
- Thrombus with in the lumen.

Thrombus formation can occur following endothelial disruption through activation of platelets through the coagulation cascade. Occurrence of large thrombus interrupting the coronary blood flow will result in MI. Genesis of occlusive intracoronary thrombus formation cannot be fully explained by atheromatous plaque rupture and thus further development of AMI. In addition to a variety of hematologic disorders, the role of the platelet-derived mediators including TXA2, serotonin, ADP, platelet-derived growth factor, tissue factor that promote an environment for thrombosis and vasoconstriction and the decreased availability of the natural endogenous substances like EDRF, tissue plasminogen activator and PGI2 occurs.

PGI2 released from vascular endothelial cells is extremely unstable and it inhibit platelet aggregation. HDL stabilizes PGI2 through

the function of ApoA-I, which resides on the surface of HDL molecules and identified as PGI2 stabilizing factor. Decrease in HDL-associated Apo A-I in patients with unstable angina and during the acute phase of myocardial infarction indicates that HDL plays an more effective role in preventing coronary atherosclerosis and intracoronary thrombus formation by stabilizing PGI2 in addition to the generally accepted biochemical property of HDL to prevent the accumulation of cholesterol by mobilizing free cholesterol from tissues or macrophages. There is also a PGI2 synthesis-stimulating factor in serum that has not yet been identified chemically. EDRF or nitric oxide provides another important regulating system in the vessel wall

## PROTECTIVE ROLE OF HDL IN MI

HDL transport cholesterol centripetally (from the periphery to the liver). Nascent HDL particles are in the form of disk shaped consisting primarily phospholipid (principally of phosphatidylcholine) &apolipoproteins A, C, and E. As HDL accumulates cholesterol they are rapidly converted to spherical particles.

## HDL is a reservoir of apolipoproteins A,C,E :

- The main apoproteins in HDL are apoAI and apoAII

- Serve as a circulating pool of apo C-II , it is transferred to chylomicrons and VLDL. Also Apo CII is an activator of an enzyme lipoprotein lipase.

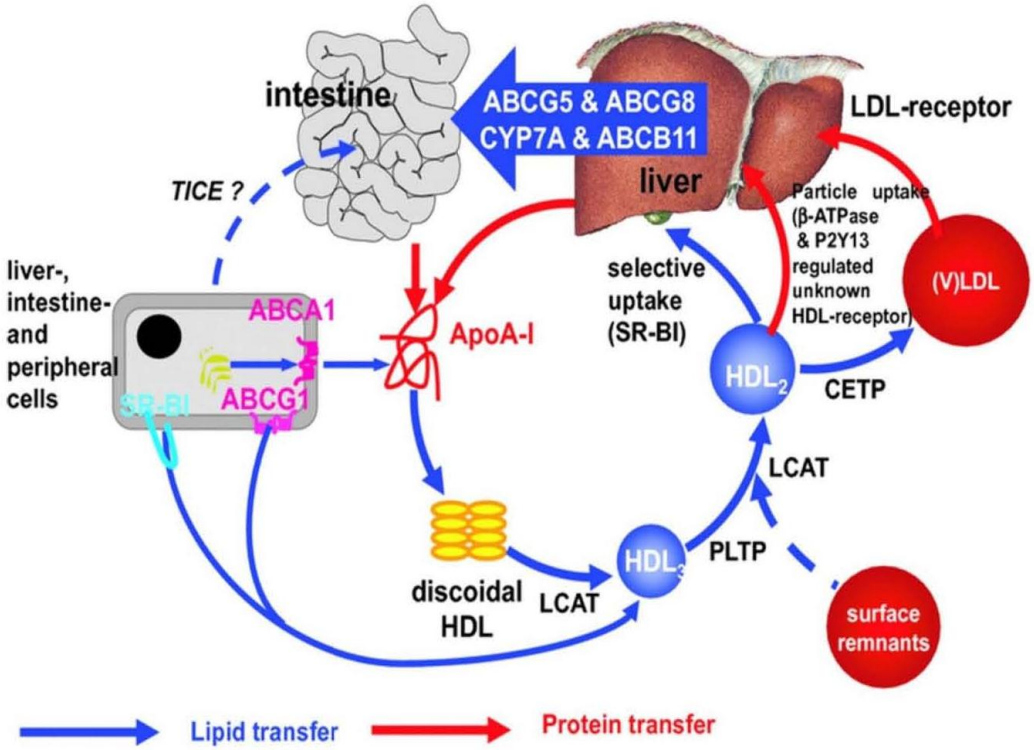
- Apo E –this lipoprotein is prerequisite for the receptor-mediated endocytosis of Intermediate Density Lipoproteins and chylomicron remnants

- Nascent HDLs are originally small ( no cholesterol) and then repetitively gain and lose cholesterol .When HDL size is compared with LDL particles , it is smaller and denser.

- Surface of each HDL particle have small number of (e.g. 2 or 3) $apoA_1$ particles and they contributes to nearly all plasma $apoA_1$[23] , vary substantially in size, cholesterol content and possibly in biological activity.

- Participates in the metabolism of other particles Chylomicrons, VLDL and remnants through component exchange ,by exchanging apoproteins, phospholipid, triacylglycerol (TGL) and cholesteryl ester .

**FIG : 6 METABOLISM OF HDL**




ester .

**HDL uptake of unesterified cholesterol:**

- HDL particles takes up the cholesterol , immediately esterifies it by the glycoprotein enzyme phosphatidylcholine: cholesterol acyltransferase (PCAT).it is otherwise known also as LCAT and L- stands for Lecithin . This enzyme is secreted by the hepatocytes.

- HDL comprises high concentration of phospholipids (PL). PL is an important solubilisers of cholesterol , this constituent in HDL make it as an excellent acceptors of unesterified cholesterol . Acceptance is from both lipoproteins particles and from cell membranes. This trail of events is known as reverse cholesterol transport (RCT)

- LCAT binds to nascent HDL, and is stimulated by apo A-I. LCAT shifts the fatty acid located at the second carbon of phosphatidylcholine to cholesterol thus yielding hydrophobic cholesteryl ester, which is impounded in the core of the HDL & lysophosphatidylcholine binds to albumin.

- As the cholesteryl esters begins to accumulate in nascent HDL, initially it transforms to fairly cholesteryl ester–poor HDL3 and finally to cholesteryl ester–rich HDL2 particle, that carries these esters to the hepatocyte.

- Cholesterol ester transfer protein (CETP), synthesised in the hepatocyte facilitates the transfer of cholesterol esters from HDL to TGL-rich lipoproteins in exchange for some of their TGL and apoproteins.

## REVERSE CHOLESTEROL TRANSPORT(RCT)

This process is carried out by HDL and its precursors [79] from peripheral tissues ( ex: artery walls). Small HDL particles (more numerous) carry only two molecules of apo$A_1$ and few cholesterol molecules, despite large HDL molecules carry three molecules of apo$A_1$ and more than 100 cholesterol molecules. Such HDL particles can transfer some of their cholesterol load to the TGL-rich precursors of LDL particles and elevated TGL levels accompany low HDL-C levels.

- The independent impact of HDL-C (particular types of HDL particle) to MI risk is therefore best evaluated by their effects on the particles or their precursors.

## HDL MATURATION AND THE PROTECTIVE EFFECT AFFORDED BY APOA-I

- Apo A-I engages the adenosine tri phosphate–binding cassette transporter A-1 protein (ABCA1) ,they retrieve the cholesterol from the peripheral cells, macrophages and they are converted into discoid pre migrating HDL.

**FIG : 7 CETP ACTIVITY IN ATHEROSCLEROSIS**

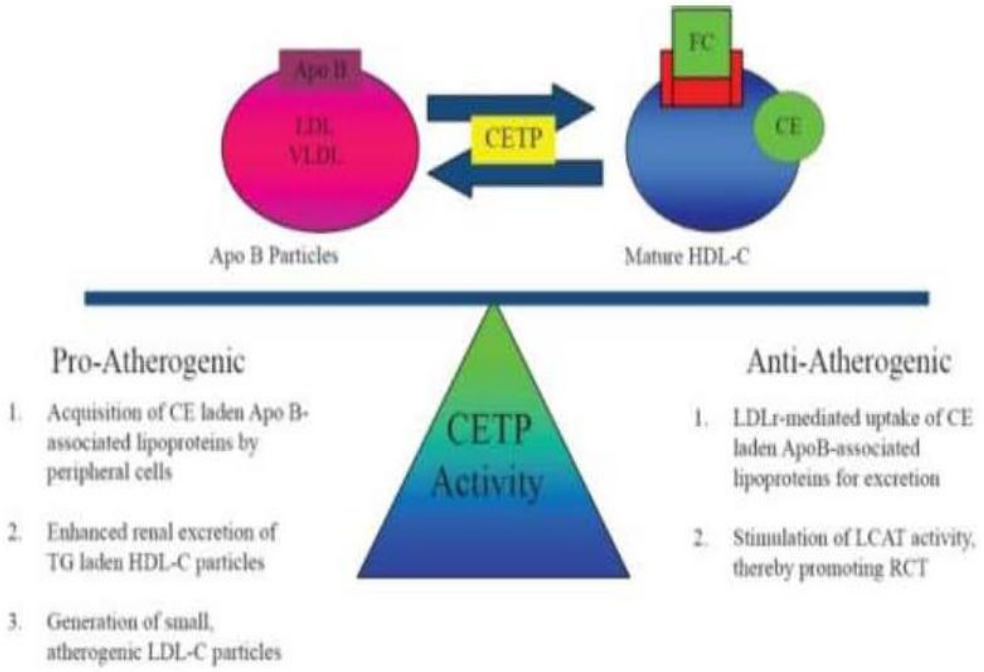
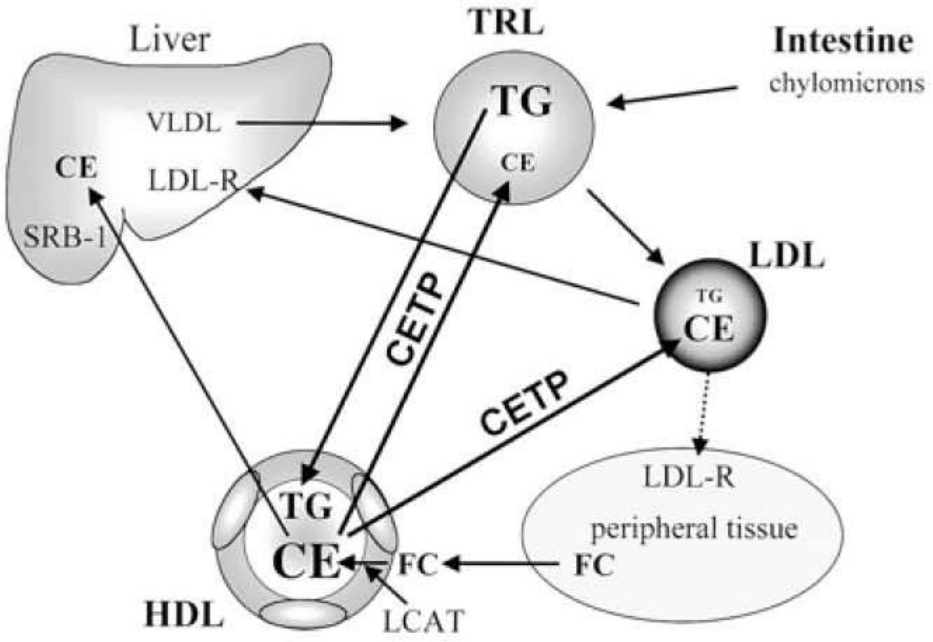



**FIG 8 : REVERSE CHOLESTEROL TRANSPORT**



- Maturation of HDL is by the action of LCAT, forming $HDL_3$ and eventually, large buoyant $HDL_2$ which is then taken up by the hepatocyte by engaging scavenger receptor B1 (SR-B1).

- Rich quantity of enzymes having antioxidant function (including paraoxonase 1-PON1) are normally found in $HDL_3$ and to a reduced amount in $HDL_2$.

- Oxidized LDL have lipid hydroperoxides, they get attached to the vascular endothelium through the lipoprotein-like receptor and stimulates vascular endothelial cells (VEC) to produce MCP-1.The Apo A1 like amphipathic 18-amino-acid peptide 4F both retrieves lipid hydroperoxides from LDL and substitutes for ApoA-I in uptake of cholesterol by engaging ABCA1, thus protects endothelial cells from ongoing inflammation .

**ROLE OF LCAT IN CHOLESTEROL EFFLUX:**

- LCAT Contains 416 AA,

  Displays two activities:

- Phospholipase $A_2$ activity-hydrolyses the *sn*-2 fatty acid from phosphatidylcholine, and

- Acyltransferase activity:transfers the fatty acid to Free Cholesterol and forms Cholesterol ester.

## OTHER FUNCTIONS OF HDL

- During bacterial infection type 1 interferon is produced ,this is prevented by HDL particle.

- Chemokines, CCL2 (MCP-1) and CCL5 are produced in the atherosclerotic plaque, this might possibly be accountable for the recruitment of cells to the affected site , such process is inhibited by HDL.

- Attenuation or inhibition of LDL oxidation,

- Stabilises enzyme endothelial nitric oxide synthase (eNOS) , this in turn causes dilatation of vessels.

- One of the fascinating feature of HDL is that it may promote insulin secretion by pancreatic islets [70]

## HDL – AS AN ANTIINFLAMMATORY AGENT

- HDL fixes to LPS, thus hinders its capacity to elicit signalling via the CD14/MD2/TLR4 complex[66].

- Recent thought is that HDL or apoA-I binding to ABCA1 on chronic inflammatory cell , macrophages is anti-inflammatory.

- One ancillary process is possibly due to lipid efflux is promoted, cell surface rafts gets reduced which leads to reduced signalling via TLR4 and possibly other TLRs.

- Alternative mechanism seems to be due to promotion of an anti-inflammatory phenotype in the macrophages by apoA-I mediated stabilization of ABCA1 resulting in the autophosphorylation of JAK2 and activation of STAT3 [66]. ApoA1 lessens the over all expression of pro-inflammatory cytokines (IL-1, IL-6, and TNFα) produced by macrophages considered to be an autonomous action of the lipid transport activity of ABCA1

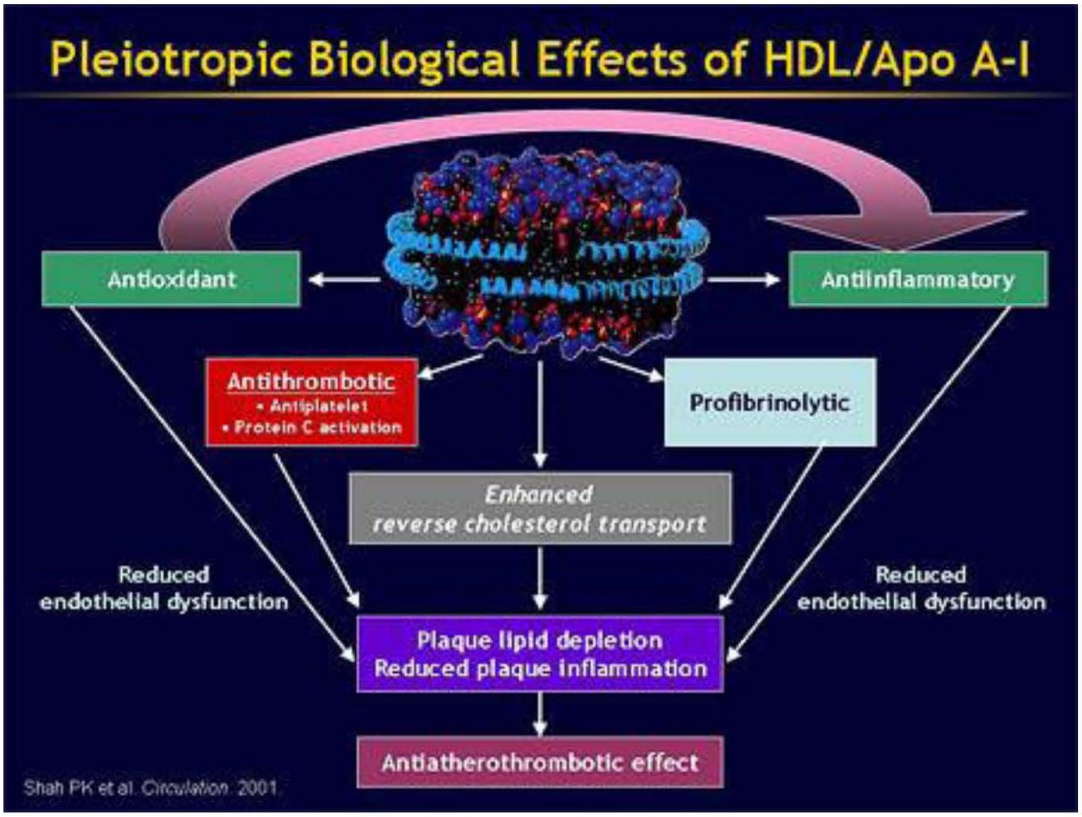
## AN ANTI OXIDATIVE ENZYME IN HDL- PARAOXONASE

- This enzyme is primarily synthesised by the hepatocytes

- Circulates in association with APO A1 and HDL

- They hydrolyse substrates such as arylesters, phosphate esters, and lactones including thiolactones.

**FIG : 9**


Pleiotropic Biological Effects of HDL/Apo A-I
Antioxidant
Antiinflammatory
Antithrombotic
• Antiplatelet
• Protein C activation
Profibrinolytic
Enhanced
reverse cholesterol transport
Reduced
endothelial dysfunction
Reduced
endothelial dysfunction
Plaque lipid depletion
Reduced plaque inflammation
Antiatherothrombotic effect
Shah PK et al. Circulation 2001

- Inactivates lipid peroxides and hydrogen peroxide and thus it offers protection against oxidative stress .

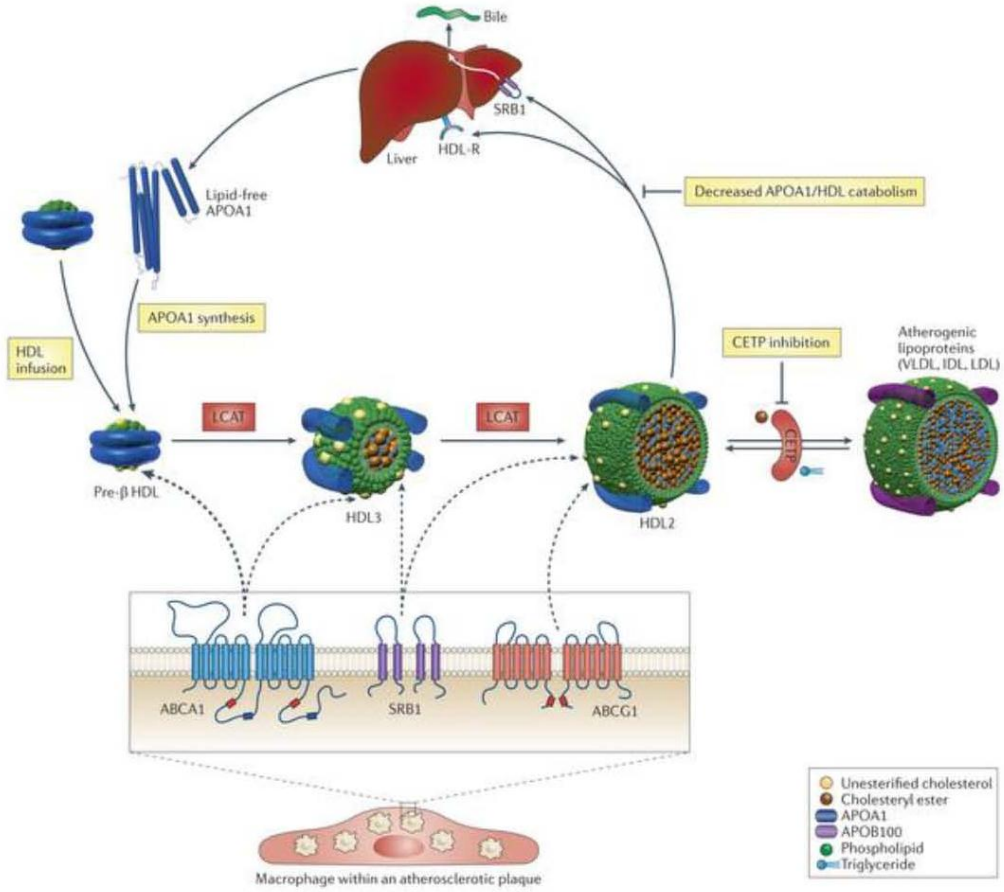
Individual mechanism by which HDL may apply this anti-inflammatory action is because of its carriage of enzymes, paraoxanase adept of cleaving oxidized Phospholipids and platelet activating factor hydrolase.

## PROMISING MECHANISMS OF THE CARDIOPROTECTIVE PROPERTIES OF APO A-I

- Augmentation of RCT, cholesterol efflux from the cells[40]
- Enriched paraoxonase activity
- Diminution of oxidative stress,
- LCAT activation
- Improved anticoagulant activity

Elucidation of the biochemical pathways regulating apo A-I gene expression is indispensable to the development of novel therapies for preventing atherosclerosis[38]

**FIG : 10 OVER VIEW HDL &APO A1 IN PREVENTING ATHEROSCLEROSIS**



## APO A1 PROTEIN

cDNA clones of Apo A1 gene was first isolated and characterised by Breslow et al . 70% of the HDL protein mass is occupied by Apo A1 approximately and 15-20% is occupied by Apo A-II. Proteomic study conducted recently shows the remaining portion of HDL protein mass is made of amphipathic proteins which includes apoCs, E, D, M, A-IV, paraxonase. Structural studies of HDL should be made on apoA-1, initially, because it occupies the bulk portion of HDL particles. Serum ApoA1 level is 1.0-1.5 mg/ml and it is a relatively abundant plasma protein. Liver and small intestine synthesizes Apo A1 as a preproapolipoprotein having 267 amino acid residue. Signal peptidase , at the time of translation cleaves 18 amino acid residues, the presegment and the product proapoA-I having hexapeptide prosegment which are in covalent linkage with the $NH_2$ terminus of mature apoA-I.ProapoA1 is then secreted into venous and lymphatic circulation and further endures extracellular posttranslational cleavage to provide matured 243-residue apoA-I[37].

Apo A1 is a solitary polypeptide chain with 243 amino acid residues of recognised primary amino acid sequence ,28-kDa single polypeptide that lacks glycosylation or disulfide linkages.

In vivo ApoA1 has multiple conformations, with metabolic interconversion between lipid-free - poor, partially lipidated and fully lipidated states, triggered by ambient lipid concentration .

Apart from the N-terminal 44 amino acids, sequence of Apo A1 is organised into eight α-helical segments composed of 22 amino acids separated by proline residues[71] . ApoA-I sequence analysis shows it is composed of repeated amphipathic α helices having 11 of 22 residues is responsible for the protein's lipid-binding regions. Hydrophobic and hydrophilic residues in the α helices of Apo A1 occupies the opposite sides of the helical cylinders. Negatively charged residues projects from the centre while positively charged residues placed at the edges and hence polar helix face exhibits zwitter ionic character. Helical segments fulfill the structural role of apoA-I, rather by the organized tertiary structure. This explains how the lipoprotein α helices float on phospholipid surfaces similar to logs on water.

Ajees .et.al first conveyed the crystal structure of lipid free human apo A1 to 2.4-angstrom resolution.[69] They revealed APOA1 consists of N-terminal 4-helix bundle with a hydrophobic core and 2 C-terminal helices. Lipid free conformation is predominantly maintained by the N-terminal domain. Hydrophobic patches formed by four leucines in the

N-terminal domain initiates unravelling of this domain to lipid bound open configuration .Function of C-terminal domain was expected to have lipid affinity and they are the sensitive trigger for the lipid-mediated unravelling of the N-terminal domain. Tyrosine in Apo A1 is chlorinated and nitrated by myeloperoxidase and this in turn causes defect in ABCA1-dependent cholesterol efflux, which is an considered to be atherogenic risk factor. Only solvent accessible area (for chlorination and nitration) in APOA1 is tyrosine-192[73].

The domain that is highly conserved and explains antigenicity (mature protein 1-98 residues)in Apo A1 is the N terminal domain and it is this domain which plays an important role in structure and function of this protein. The central and C-terminal domains show conservative substitutions between species.

- Binding of lipids is by the N and C terminal domains of Apo A1- important function of this protein
- LCAT activation is by the central domain- suggests that evolution might have occurred parallel.

## STRUCTURE OF LIPID-FREE APOAI :

- Stability and solubility of ApoA1 in lipid free state is primarily by the α-helices organised in bundles near the N-terminal half (AA 44-126) where they are loosely folded .

- Lipid poor Apo A1 mobilises cellular free cholesterol and phospholipid , is by the unstable C-terminal half (AA190–243).This area provides stability to α-helical segment including self-association and lipid binding.

- Function of the central region in the Apo A1molecule between residues 139–170 is interaction with the N-terminal portions thus stabilizing the α-helical bundle in the lipid-free state.

- Negative potential field around apoA-I is responsible for the docking of lipid-free apoA-I with ABCA1.

## STRUCTURE OF LIPID-BOUND APOAI

- Major stabilising domain is the C-terminal half in lipid bound state and it becomes more highly α-helical.

- Protein lipid contacts is made by the rearrangement of helixes involved in protein –protein interaction in the N terminal of Apo A1

## TERTIARY STRUCTURE OF APO A1 IN LIPID FREE &BOUND FORMS FOR DISCOIDAL LIPOPROTEINS :

Two models were proposed for two apoA-I molecular arrangement with phospholipids. [72]

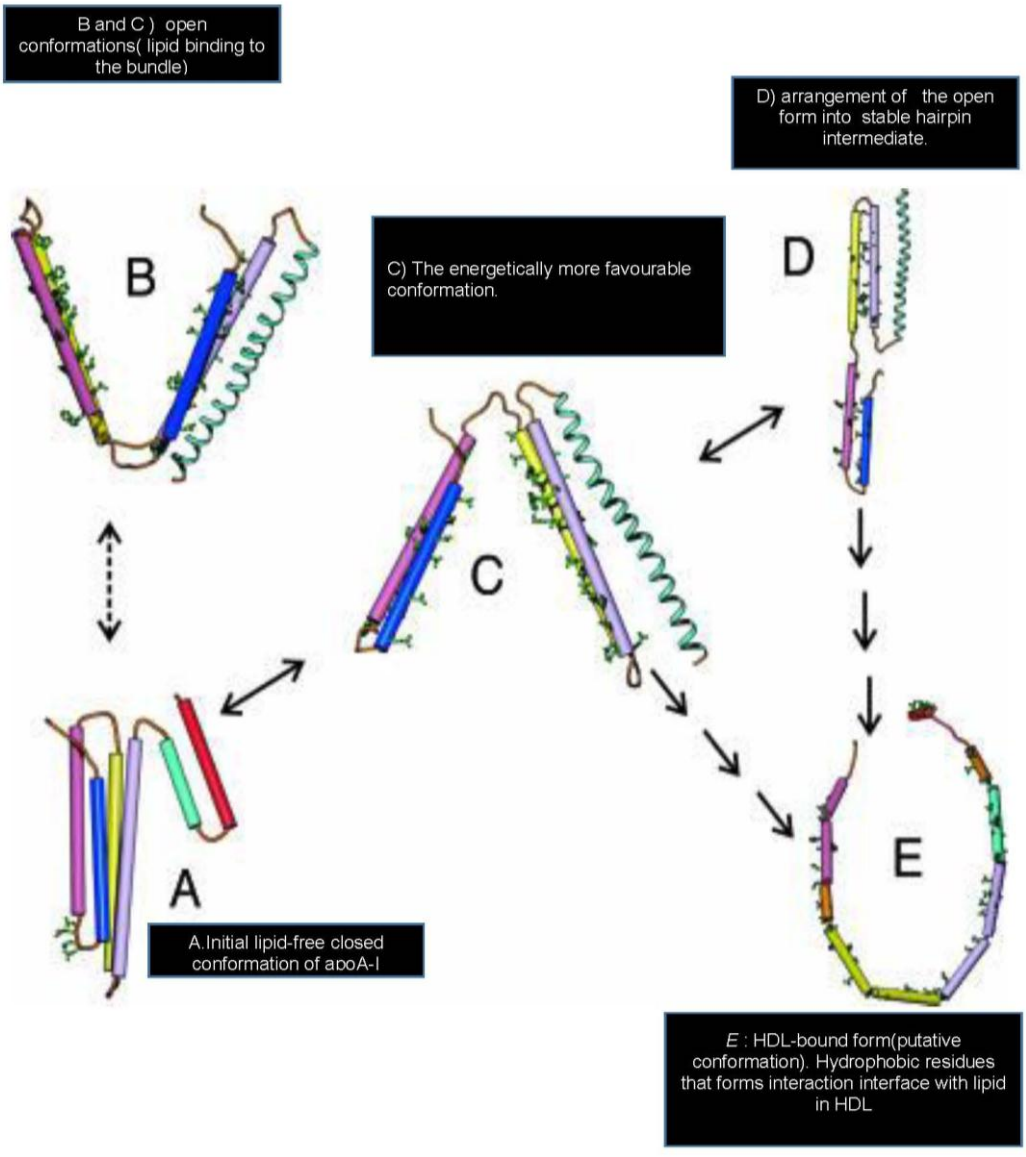
I. In Picket fence model: Individual molecule of apoA-I wraps around the HDL disc in an antiparallel fashion with little intermolecular interactions between adjacent alpha-helices.

II. Belt model: Two antiparallel apoA-I particles are paired by their C-terminal alpha-helices, wrap around the lipoprotein, and are stabilized by multiple intermolecular interactions. Recent evidence supports the belt model.

ApoA-I alpha-helices control lipid binding and association with varying levels of lipids.

- LCAT activation domain : Clearly assigned to helix 144-165 with secondary contribution by helix 166-186.
- Displacement of apoA-I helices by LCAT and presentation of the lipid substrates : By the lower lipid binding affinity of the region 144-186 .

## FIG.11 LIPID FREE - LIPID BOUND STRUCTURE OF APO A1



- No specific sequence has been found that affects diffusional efflux to lipid-bound apoA-I.

C-terminal helices : important for lipid binding and maintenance of HDL in circulation, interaction of lipid-free apoA-I with macrophages and specific lipid efflux.

## MECHANISM BY WHICH CHOLESTEROL EFFLUX IS MEDIATED BY APO A1:

Desorption of free cholesterol from the plasma membrane is the rate limiting step in diffusional efflux

Factors which influences Desorption depends on

- Properties of the cell membrane
- The cell type in which it occurs
- Presence of diverse pools of cholesterol &their inter-exchange with the plasma membrane
- Protein distribution of HDL(Ratio of apoA-I &A-II )
- Key factor may be the lipid composition of acceptors.

Cholesterol flux between lipoproteins and cells is influenced by Free cholesterol and phospholipid content of HDL.

## ABCA1 RECOGNITION MOTIF ON APO A1

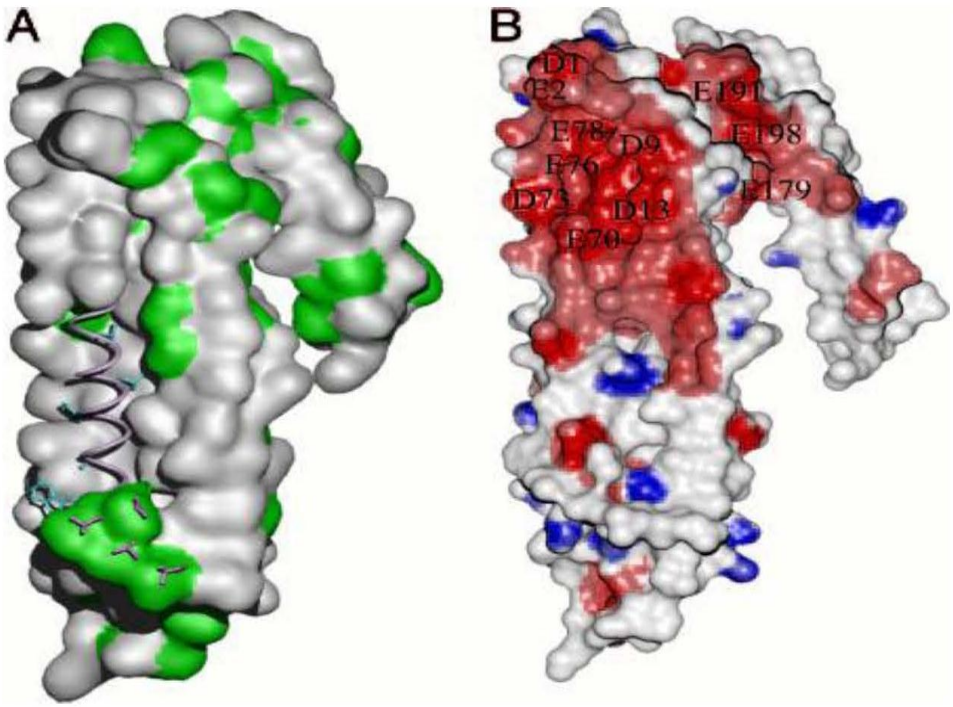
Prior to cholesterol acceptance it is the docking of Apo A1 with ABCA1[73], this process is not sequence specific and these have proven in recent studies. Surface charge analysis reveals that there exists an interaction between apoA-I with ABCA1 and SR-B1. ABCA1 recognition motif on apoA1 is the dominant two negative patches. C terminal patch which is hydrophobic in nature adjacent to negative charge cluster, is the site where ABCA1 interacts with lipoproteins. Aspartic acid and glutamic acid at position seventy three and seventy eight respectively belonging to helix two, contributes to largest negative patch which is ≈28Å apart from the hydrophobic patch on C terminal domain is the ABCA1 recognition motif, although it is not proved with certainty. Negative potential field is created by the preponderant negative charge. Important prologue to is interaction is the electrostatic attraction of apoA-I with positively charged regions of ABCA1[73].

## SR-B1 RECOGNITION SITE ON APO A1[73]

SR-B1 has separate binding sites for both lipid free and HDL-associated apoA-I.

**FIG.12**

## SURFACE HYDROPHOBIC & ELECTROSTATIC AREAS OF APO A1




A: Gray: Solvent accessible , green : Hydrophobic patches , purple shade : they are the side chains of leucine superimposed on the patch at the base of helix

B: Positive ( shaded blue) and negative electrostatic potential (shaded red) mapped to the molecular surface of apoA-I, oriented to show the largest negative patch.

Synthetic peptides analysis point out that the hydrophilic face of apoA-I helices is recognized by SR-B1. The surface of apoA-I has various potential positively charged docking regions for attachment to SR-B1. Offloading of cholesteryl ester to the hepatocyte is facilitated through contact of HDL bound apoA-I with SR-B1, whose extracellular domain binds both HDL and LDL.

## COMPREHENSIVE VIEW OF POLYMORPHISM & APO A1 GENE

### POLYMORPHISM[36]:

Otherwise known as polymorphic allele.

- Defined as the occurrence of natural variations in a chromosome, DNA sequence or gene without inflicting any adverse effects on the individual.

- In general population it exist in fairly higher frequency.

It could be of either single base/nucleotide pair variation (SNP) or it involves long stretches of DNA of which the first type is the most common one.

## SINGLE NUCLEOTIDE POLYMORPHISM

- Alternative term “snips”.
- Most of the SNPs have no issues on health or development.
- On an average occurrence of SNPs in DNA is 1 / 300 nucleotides.

## SIGNIFICANCE BEHIND SNP STUDY

- SNPs as biological pointers, it helps inventors to locate candidate genes that are linked with disease.
- Genes function can be affected if SNPs occur inside a gene or in a regulatory region proximate to a gene.
- Disease that run through families can be traced using SNPs.
- Susceptibility to toxins , particular diseases and person’s reaction to certain medications can be predicted by SNPs
- Future scope of SNPs study is its association with complex diseases such as heart disease, diabetes, and cancer.

## APO A1 GENE DESCRIPTION AND ITS REGULATION

The reason behind why the Asian immigrants and Indians in urban area are at increased risk of developing MI is not yet clear. To understand the wide range of genetic/epigenetic influences on CAD[30] requires systematic and comprehensive study. Holistic approaches are needed to find the multiple factors (including genetic and environmental )behind the atherosclerosis, which eventually leads to MI.[26-29] Wide variety of candidate genes was investigated behind the evolution of CAD[26-28] which includes APOE, APOB, LPL, iNOS, ACE, COX2, CD14, P-Selectin, ESelectin, MTHFR, PON1, TNFα . Only few studies on these genes have come out with promising signals to play a role in CAD.

**HGNC APPROVED GENE SYMBOL: APO A1Apolipoprotein A-I**

Short name=Apo-AI; ApoA-I

**Alternative name :**

Apolipoprotein A1

Cleaved into the following 2 chains:

1. ProapolipoproteinA-1

   short name=proapoA1

2. Truncated apolipoprotein A-1

Taxonomy ID: 9606 (NCBI)

Gene ID: 335

Genbank common name: **human**

Inherited blast name: **primates**

Gene Type : Protein Coding

Rank: species

Authority: Homo sapiens Linnaeus,175

## CYTOGENIC LOCATION

**Ensembl cytogenic band : 11q 23.3**

**Entrez gene cytogenic band: 11q 23- q24**

**HGNC cytogenic band: 11q23-q24**

**Exon count: 5**

**Annotation release (current) : 106**

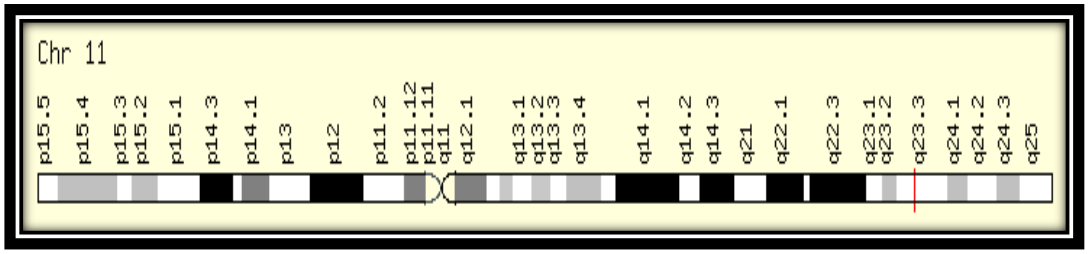
**(previous) : 105**

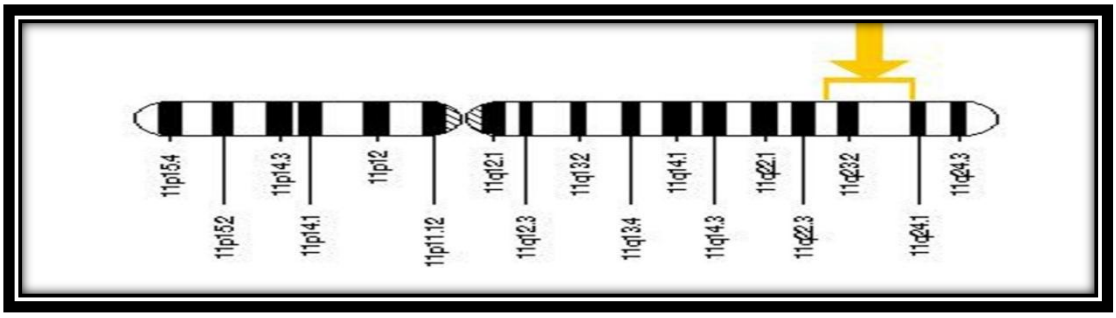
**Genomic coordinates:**

**(GRch38):11:116,835,751-116,837,950**

**(GRch37):11:116,706,466-116,708,337**

**Approximate molecular weight of 28.1 KDa**

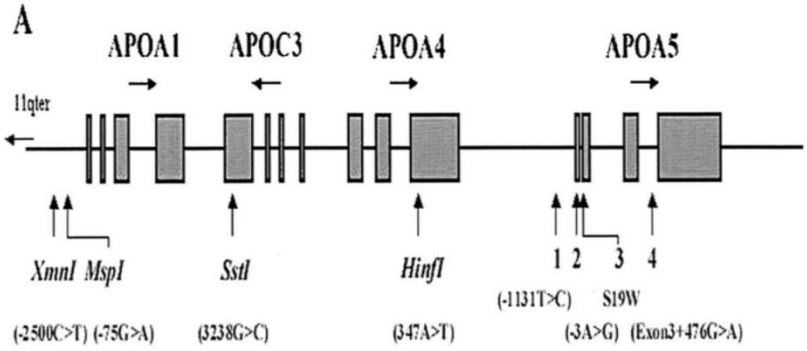





## APO A1, CIII & AIV GENE COMPLEX - FROM COMMON EVOLUTIONARY PRECURSOR

The APOAI gene is coded on the chromosome 11 along with APOCIII and A IV genes. The APOA1-CIII-AIV genes are interlinked[32-35] and this cluster is approximately fifteen Kb. Transcription of Apo C-III gene is in the reverse direction to Apo A-I &A-IV. Foot-to-Foot orientation is seen with APOA1 and CIII genes(3-prime end of APOA1 is trailed after an break of about 2.5 kb by the 3-prime end of APOC3)[1].Clusters that contains APOA1-CIII-AIV genes thought to have been evolved from the same ancestral sequence, has its dominant role in metabolism of lipid and lipoproteins. Apo A1 & Apo CIII has three introns , apo A IV has only two introns. Intron interrupting the 5' noncoding region of mRNA of Apo A1 & Apo C III is absent in Apo AIV .Any genetic variation within this gene cluster will have a significant influence on the hepatic & intestinal regulation of ApoAI-C-III -ApoA-IV gene expression. Amino acid sequence analysis studies on apoproteins A-I, A-II, C-I,

and C-III says that they all would have derived from a common evolutionary predecessor. Apo A1 gene after its separation was stretched out by internal gene duplications. This contributes to six homologous 22-amino-acid long structures which inhabits most of the COOH-terminal half of the apo A-I polypeptide.




## APO A1 GENE DESCRIPTION:

- Apo A1 gene is interposed by three intervening sequences (IVS) , IVS-1, IVS-2, and IVS-3 happening in the 5'noncoding region of apo A-I mRNA, the mRNA sequence coding for the signal peptide of apoA-I,[39] and the sequence coding for the mature protein, respectively.

- The existence of interposing sequences in the DNA region coding for the signal peptide, proposes that introns may have aided at some initial time to bring different exons together.

- Finally,the polyadenylation signal and its sites of apo A-I mRNA are located 36 bp and 55 bp, respectively downstream from the TGA terminator of the apo A-I gene .

- The coding region of the mature apo A-I mRNA, AUG initiator and UGA terminator offers the complete amino acid structure of the primary translation product of apo A-I mRNA.

- Proteolytic cleavage between the amino acids at positions -1 (glutamic acid) and + 1 (aspartic acid) is required for the conversion from Proapo A-I to mature apo A-I .

- The sequence "TATA" (TATA box) has been perpetually found 25-30 bp upstream of the mRNA start sites of many eukaryotic protein coding genes and appears to play a role in the elevation of transcription.

## DEFECTS IN APO A1 GENE ARE ASSOCIATED WITH HDL DEFICIENCIES

- Tangier disease - Either due to an alteration in the coding region around the glutamine (- 1)-aspartic acid (+ 1)site of apo A-I gene or to a different mutation that affects the conversion of proapo A-I to mature protein[1]

- Systemic non-neuropathic amyloidosis.

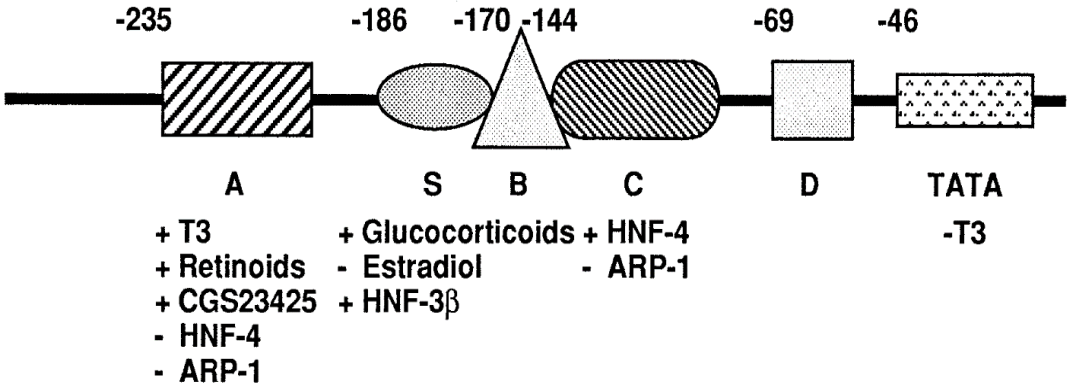
## APO A-I GENE REGULATION

APO A1 gene has been used as a model for the tissue specific expression in hepatocyte and intestine. The term tissue-specific genes are, in general, methylated other than the expressing tissue.[75]It is the 5' region methylation status of ApoA1 gene determines the tissue specific activity. In all the tissues the 3'-end flanking sequences are invariably hypomethylated due to an unusual characteristic CpG island at the 3'-end coding sequence. Regulation of Apo A1 gene expression is at the transcription level and is influenced by various factors like hormone, diet,developmental,physiological and various environmental stimuli. The promoter region of ApoA1 gene has TATA-like motif closer to the transcriptional start site .Alteration in hormonal or metabolic status can affect the expression of Apo A1 gene in a progressive or deleterious manner due to the existence several cis regulating elements

Hepatocyte - specific Apo A1gene expression is chiefly governed by four families of liver-enriched transcription factors(TF): They are

1) Hepatocyte nuclear factor 3 (HNF3)
2) Hepatocyte nuclear factor 1 (HNF1)
3) The CCAAT enhancer binding proteins (C/EBP) family and
4) Various members of the nuclear receptor superfamily, such as hepatocyte nuclear factor 4.

Hepatocyte -specific genes are regulated by promoter/enhancer sequences containing closely spaced *cis*-acting elements on which various combinations of hepatocyte-enriched and ubiquitous factors assemble [58]. The gene which codes for the apoAI, contains a powerful liver-specific enhancer present in the region between –222/–110 nucleotide upstream of the transcription start site.Three cis-acting elements A(–214 to –192),B(–169 to –146)and C(–134to -119) maintain the ApoA1 gene transcription in the liver with in the enhancer region .Sites A and C are bound by members of the nuclear receptor superfamily, including HNF4, ARP-1, RXRa and RAR/RXR heterodimers. Site B binds the hepatocyte-enriched factors HNF3ß, HNF3α and C/EBP . These factors although they bind independently to their cognate sites, they stimulate apoAI enhancer activity synergistically via conjoint recruitment of an uncharacterized transcriptional coactivator(s)



Synergy may result from cooperative binding of the transcription factors to their respective cognate sites. Alternatively,multiple secondary interactions of enhancer-bound factors with targets in the core transcription machinery could result in transcriptional synergy .The latter could occur directly, involving components of the RNA polymerase II-specific basal transcriptional factors (including the TATA box-binding protein-associated factors ;or indirectly, through adaptors and/or coactivators that serve to bridge the enhancer and core complexes.

When both HNF-3 and HNF-4 occupy their binding sites, an intermediary factor that is present in hepatocytes is recruited to the enhancer and core transcription complexes , but not with either of them occupying their binding sites individually. By this way, this factor, in addition to simply being an adaptor molecule, could also function to integrate the signals regulating the primary TF (HNF-3 andHNF-4).This observation also adds another level at which apoAI tissue restriction is enforced. Thus, it is not expressed in some extrahepatic tissues because they lack the primary transcription factors, and in others, because the intermediary factor is absent.

## REGULATION OF APO A-I GENE – SUBJECT TO ALTERATIONS BY SEVERAL HORMONE & METABOLIC SIGNALLING PATHWAYS

Its been noted various hormones stimulate apo A-I gene transcription. They bring apo A-I promoter action along with gene expression via proximal promoter elements resides between the nucleotides -235 and -144 (relative to the transcriptional start site, + 1)

- Thyroid hormones &Retinoids – Directly through their nuclear receptor which act on Apo A1 promoter[58]

- Estradiol – Inactivity of the estradiol-responsive transcriptional corepressor RIP- 140, by the interaction of HNF-3ß and the orphan nuclear receptor HNF-4 on the apo A-I promoter[58],thus increasing apo A-I gene transcription.

- Action of Glucocorticoids on Apo A1 gene – They stimulate the binding of transcriptional activator (HNF) - 3ß to the Apo A1promoter

## APO A1 GENE FACTORS CAUSING TRANSCRIPTION REPRESSION:

- HNF-4
- Thyroid hormone receptor -Thyroid hormone receptor monomer fixes to a negative thyroid hormone response element (nTRE) existing in the 3' of the apo A-I gene TATA-box may destroy apo A-I gene transcription .
- Insulin Response Core Element- (IRCE)Positioned between nucleotides - 404 and – 411 which is in 5' from the transcriptional start site. This element fixes to the ubiquitous TF, Sp1 is liable for the beginning of the apo A-I gene expression by insulin.
- ARP-1 - apo A-I repressor protein-1

## ACTION OF INSULIN ON THE APO A-I PROMOTER THROUGH AT LEAST TWO SIGNALING PATHWAYS

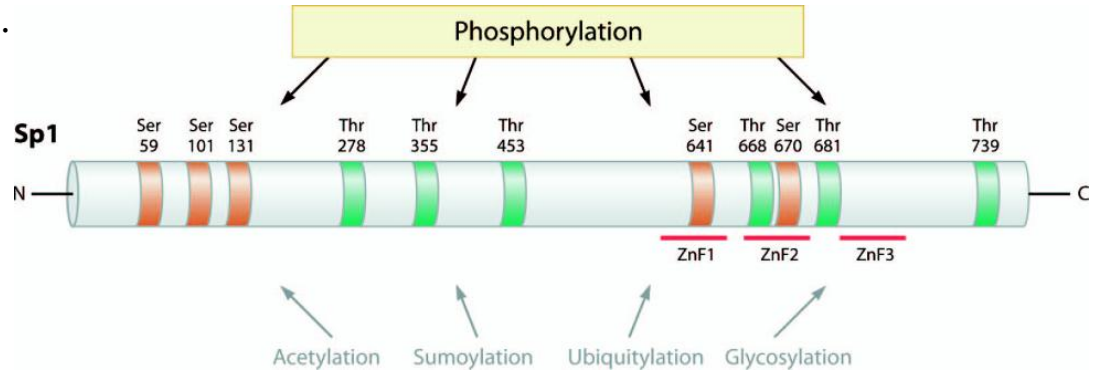
Ras-raf and phosphatidylinositol 3-kinase (PI3-K), primary to activation of the mitogen-activated protein kinase (MAPK) and PKC(kinase C) kinases, correspondingly these pathways eventually target the TF Sp1[59,60]. Phosphorylation of Sp1 shows a vital role in apo

A-I expression by regulating the ability of Sp1 to bind the IRCE. Copious domains at Sp1 are targets for phosphorylation by multiple protein serine/threonine kinases.

Sp1 has amino acid motifs recognized by both kinases. several potential phosphorylation sites for a variety of protein kinases including cAMP- or cGMP-dependent kinase, PKC, mitogen-activated protein kinase (Erk), casein kinase .Recent studies suggest that Sp1 is phosphorylated by Erk2 PKC , casein kinase or PKA The phosphorylation modification of Sp1 protein may lead to either an increase (*e.g.*Erk2) or decrease (*e.g.* casein kinase II) in its DNA binding activity. The stimulatory effects of these kinases on apoAI promoter may require phosphorylation of Sp1[77]. GC-rich sequences can bind to more than one member of the Sp family.

Sp1 phosphorylation at Thr355 activates the ApoA1 promoter.

.



Adrenalin and glucagon, activate hepatocyte adenylyl cyclase, and therefore potentially augment apoAI gene transcription, are traditionally thought of as agents that are "counter-regulatory", in the case of apoAI, the effects of insulin would be in parallel or additive with the effects of glucagon and adrenalin. Similarly, by activating Gαq, hormones such as noradrenalin (α1-adrenoreceptor) and angiotensin-II (AT1 receptor) would activate PKC and add yet a further boost to apoAI expression.

## EFFECT OF INSULIN RESISTANCE ON APO A-I EXPRESSION

Reduced plasma concentration of HDLc and low plasma levels of apo A-I is one of the hallmark of insulin resistance or the metabolic syndrome X. It is contributed by augmented fractional clearance of HDL without an alteration in apo A-I production [62,63]. Reduced responsiveness of the apo A-I gene to insulin perhaps reduces the apo A-I gene expression .

## APO A-I GENE EXPRESSION VS ALTERATION IN MICRONUTRIENTS & VITAMINS STATUS

Either insulinomimetic/permissive effects on insulin action: By minerals which includes chromium, vanadium, magnesium and zinc.

**ZINC DEFICIENCY :**Transcription factors has zinc finger motif (in Sp 1) and hence it reduced control apo A-I gene expression[58,65].This describes the organising effect of zinc ions

## CONCENTRATIONS OF ZINC, CHROMIUM OR VANADIUM AT SUPRAPHYSIOLOGICAL LEVEL

Above mentioned minerals in increased concentration downregulates the apo A-I promoter activity. Vitamins having antioxidative action may disturb apo A-I gene expression. oxidative state of a cell is also the trigger to the Apo A1 promoter activity.

## ENVIRONMENTAL VARIABLES AND ITS EFFECT ON APO A1 GENE EXPRESSION

The most important environmental variables includes smoking, fat content in the diet, body mass index (BMI) and exercise . Oxidative stress caused by these environmental variables may act by selectively reducing liver apoA-I mRNA level[68] increase in apoA-I mRNA degradation and a 2-fold rise in transcription of apoA-I gene.

## UPREGULATION OF APO A1 GENE BY PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR α (PPAR- α )

Vu dac et.al showed that Apo A1gene transcription is induced by interaction of PPARα with the positive PPRE which is present in the A site of Apo A1 gene promoter liver specific enhancer region. It also upregulates Apo A1, A II , HDLs surface phospholipid transfer protein and SR-B1,situated in hepatocyte outer membrane. Expression PPARα is high in brown adipose tissue and hepatocyte and to a smaller extend in Renal tissue, Cardiac &Skeletal muscle.

Three PPAR subtypes are : α, γ and δ/β . They show a tissue-specific expression and its functions overlaps with each other and even with that of other NRs .

Some ligands such as polyunsaturated fatty acids and oxidized fatty acids are shared between the three subtypes. These ligands are able to infiltrate the cellular membranes and reach their target receptors in the nucleus.

Regulation of Apo A1 gene is by activation of PPAR-α[80]either directly by ligand or by ligand binding to RXR thus forming heterodimers.The natural RXR ligand, 9-*cis* retinoic acid, as well as synthetic RXR-selective compounds can trigger a PPRE– driven reporter gene in a PPAR-RXR-dependent manner .[55]

## FIG.13 ROLE OF PPAR α IN FAT METABOLISM

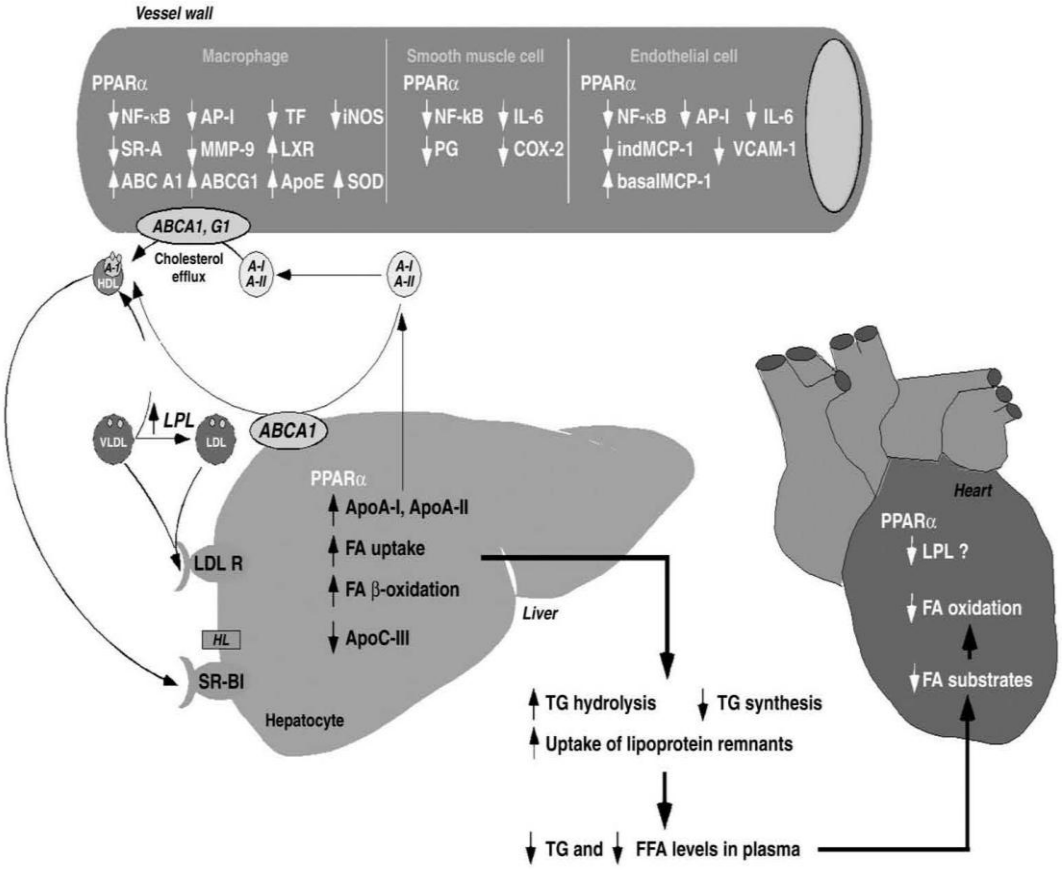
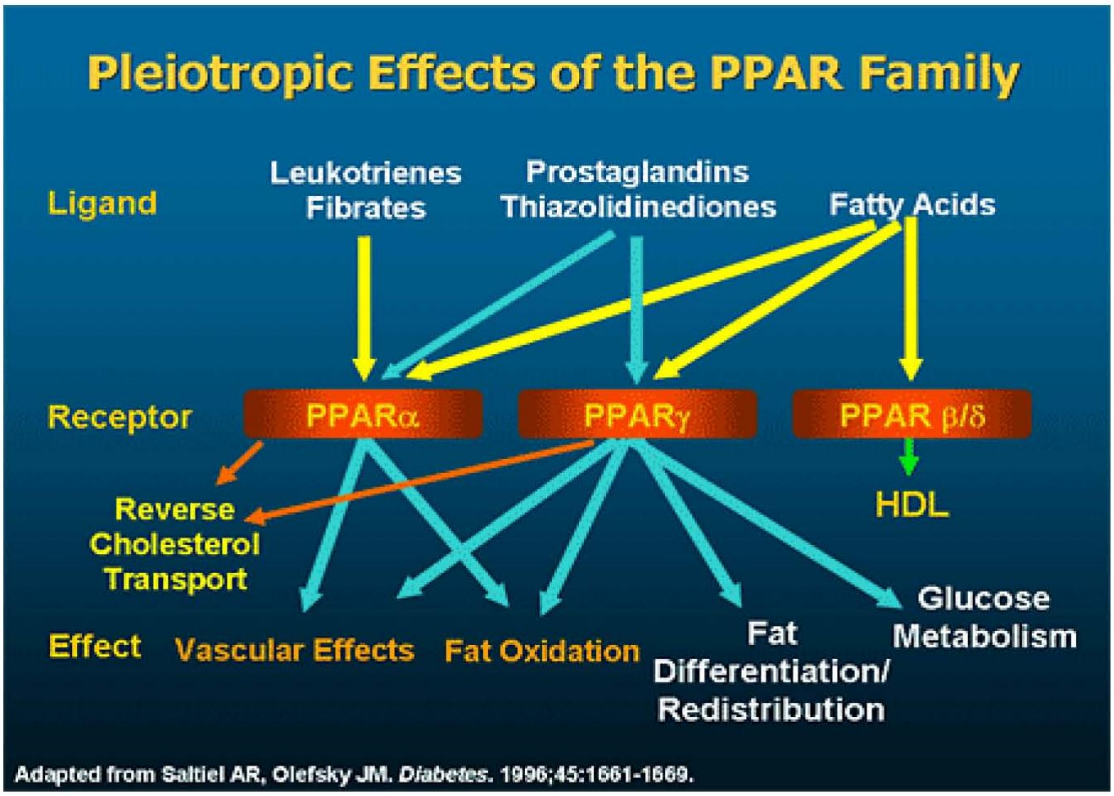


**FIG.14**


Pleiotropic Effects of the PPAR Family
Ligand
Leukotrienes
Fibrates
Prostaglandins
Thiazolidinediones
Fatty Acids
Receptor
PPARα
PPARγ
PPAR β/δ
Reverse
Cholesterol
Transport
HDL
Effect
Vascular Effects
Fat Oxidation
Fat
Differentiation/
Redistribution
Glucose
Metabolism
Adapted from Saltiel AR, Olefsky JM. Diabetes. 1996;45:1661-1669.

## PPAR RESPONSE ELEMENTS

Transcription is regulated by PPARs by forming heterodimers with RXRs. This receptor dimer is found to bind with REs occupying the 5'-hexamer.

## PPAR s –AN ADOPTED ORPHAN NUCLEAR RECEPTOR[43,44,45]

PPARs comes under the family of adopted orphan nuclear receptors. Ligands have been identified for these orphan receptors and they are low-affinity dietary lipids like cholesterol derivatives and bile acids .They bind DNA as heterodimers with retinoid X receptor (RXR).

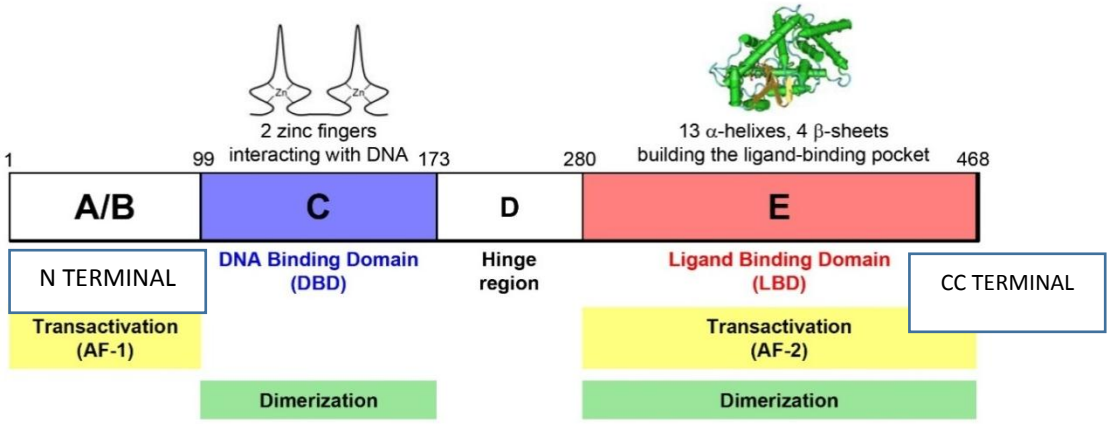
Other members of this group are as follows:

- Bile acids through farnesoid X receptor
- Fatty acids oxysterols through liver X receptor
- Constitutive androstane receptor &
- Xenobiotics via pregnane X receptor

## COMPREHENSIVE VIEW OF ALL THE ABOVE MENTIONED TRANSCRIPTION FACTORS(NUCLEAR RECEPTORS) AND ITS MODULATION OF TRANSCRIPTION

Nuclear receptors (NR) are TF, plenty of which are ligand triggered and they control the expression of precise target genes

involved in reproduction, development and general metabolism and is considered to be essential [46]. Also some unexpected ligands like glucose for the liver X receptor and dietary lipids having low affinity for PPARs .When NRs are in the nucleus and attached to DNA, they curb transcription by recruiting coregulators and components of the basal transcriptional machinery.




## STRUCTURE OF NUCLEAR RECEPTOR

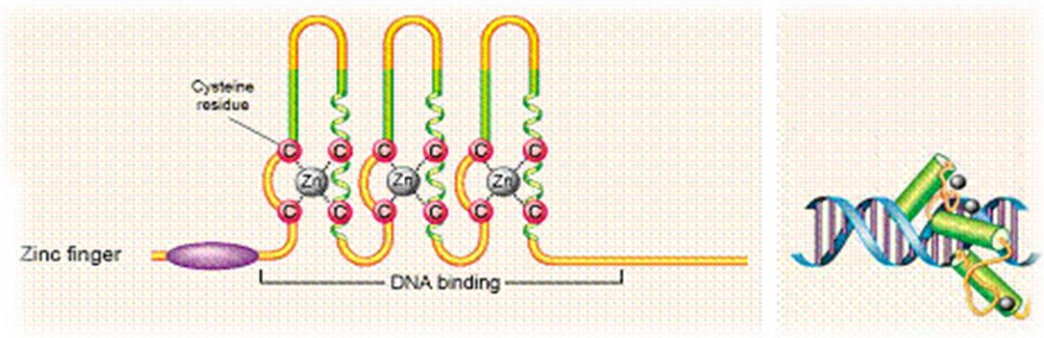
NRs contain a highly preserved DNA-binding domain (DBD) &ligand-binding domain (LBD)

The flexible N-terminal domain contains in most NRs

- Ligand-independent transcription activation function (AF-1) [53.] .
- N-terminal and linker (hinge) areas of NR proteins are not conserved.

The interaction with CoAs and CoRs is controlled via helix.

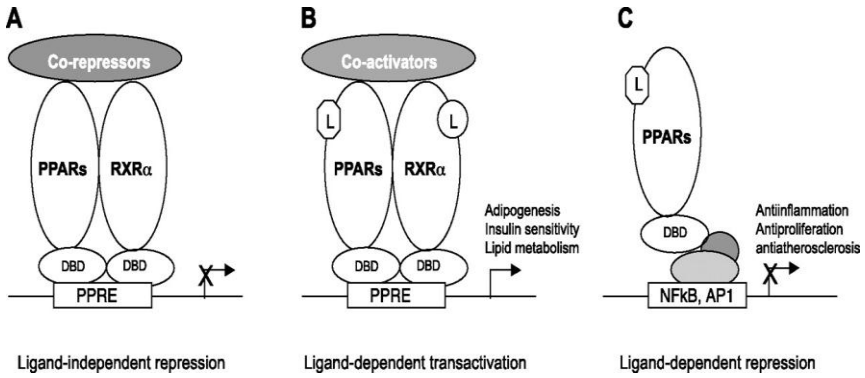
NRs bind DNA through zinc-finger motifs (zinc-finger C4-type) in their DBD. The DBD of all NRs contains two α-helices perpendicular to each other [54].




One α-helices is located behind the zinc finger and is inserted into the major groove of a DNA sequence. Since the recognition helix is highly conserved throughout the NR family, almost all NRs recognize common DNA sequences

## TRANSCRIPTIONAL SILENCING – NR WITHOUT LIGAND:

Recruits transcription-repressing factors known as CoRs to the target gene promoters[47.] CoRs, such as nuclear corepressor (NCoR), recruits histone deacetylases (HDACs) , ATP-dependent remodelling complexes (ADCRs), such as the nucleosome remodelling and histone deacetylase complex (NURD), are also recruited by NRs in their repressive state to silence transcription by affecting chromatin structure in an ATP-dependent manner [49]. Together these factors create a chromatin environment that actively reduces transcription.




## TRANSCRIPTIONAL ACTIVATION – NR WITH LIGAND:

When ligand binds to NR, it experiences conformational change, which then indicates to CoR abolishment and CoA recruitment . [50] CoAs can affect in different ways. Certain CoAs form a protein bridge between TFs and the basal transcription machinery allowing the communication between the distal enhancer elements and the proximal promoter[51]. TFs play an vital role in gene expression.

## INTESTINAL REGULATION OF APO A1 GENE[61]

Orphan nuclear receptors HNF-4 –when over expressed in intestinal cells induces Apo A1 gene expression. Expression of the apoAI gene in hepatocyte and intestine is organised by separate and distinct tissue-specific apoAI promoter regions, the activity of which is further induced by a distal enhancer located within the nearby apoCIII promoter.HNF-4 appears to play an important role in the enterocytic but not hepatic expression of the apoAI gene.

# AIM OF THE STUDY

Apo A1 shows a main part in preventing the evolution of atherogenesis which is the main causative event in MI . A large number of genetic studies were conducted to find the association of various Apo A1 gene polymorphisms and susceptibility of MI in different populations. With this background, the candidate gene of this study is Apo A1gene at two Msp 1 restriction sites **G–75A transition in the promoter region and C+83T transition in the first intron.**

Aims of the study were,

1. Is there any genetic polymorphism in Apo A1 gene at two Msp 1 restriction sites**G–75A transition in the promoter region and C+83T transition in the first intron** in the studied population?

2. To study the distribution of the Apo A1 polymorphic allele

3. To find the association between the above mentioned two Msp 1 restriction sites with respect to MI & Plasma lipid profile.

# MATERIALS AND METHODS

This study was carried out during the period March 2014 – August 2014. It is a case- control study. The study population comprised of MI patients admitted in Cardiology Intensive Care Unit, Kilpauk Medical College Hospital, Chennai. All procedures concerning human subjects or patients were permitted by the Institutional Ethical Committee.

## STUDY POPULATION:

## CASES:

The study group consisted of 52

- Documented MI patients who got admitted in cardiology ICU
- Older than 40 years
- Either gender

Patients were selected randomly who got admitted to the hospital and who fulfilled the inclusion criteria.

## SELECTION CRITERIA FOR MI CASES INCLUDED:

- Ischemia symptoms
- Pathological Q waves traced in Electrocardiography

- New or recognised significant ST-segment–T wave (ST–T) changes
- Occurrence of New left bundle branch block (LBBB).
- ECHO findings of new loss/ regional wall motion abnormality of viable myocardium

**CONTROL SUBJECTS:**

The control group consisted of 52 numbers.

Controls were recruited from outpatient clinic, Kilpauk Medical college.

**SELECTION CRITERIA**

- Age, Gender and Risk factor matched .
- No history or clinical evidence indicative of CAD.

**EXCLUSION CRITERIA**

- Patients with Liver disorder.
- Patients with Renal disorder.
- Patients with Thyroid disorder.

All cases and controls were subjected to detailed history with special reference to cardiovascular disease risk factors followed by complete clinical examination.

## SAMPLE COLLECTION

For the study, 5 ml of 12 hours Fasting Venous Blood was collected under sterile conditions from the ante cubital vein with explicit informed consent .

From the cases, the sample was collected within 24 hours of the episode of MI.

I. 3ml of blood was collected in plain vials and serum was separated after centrifugation at 3000 rpm for 10 minutes and alliquoted, into 3 eppendorfs and stored at -20°C and were not thawed until the batch was analyzed for extended Lipid profile and routine chemistry examinations

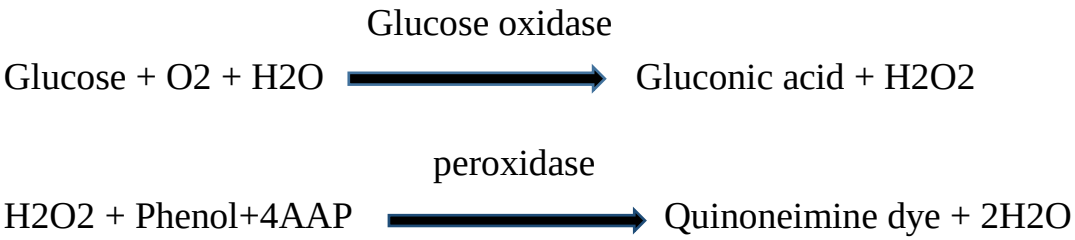
1. One aliquot was used for glucose, urea, creatinine and
2. One aliquot was used for lipid profile estimations
3. One aliquot was used for apoB and apoA1 estimations

## ESTIMATION OF FASTING SERUM GLUCOSE

**Method:** Glucose oxidase peroxidase (GOD/POD)

**Kit Used: Erba**

**Principle**




The intensity of pink colored Quinoneimine dye is proportionate to glucose concentration and was measured at 505nm.

**Reagent Composition**

Reagent1 : Enzyme reagent

Glucose standard-100mg/dl

**Procedure**

To 1ml of working solution, 10µl of plasma was added and incubated at 37°c for 5 minutes and absorbance was measured at 505 nm.

**Reference range**

Fasting plasma glucose: 70 –100 mg/dl

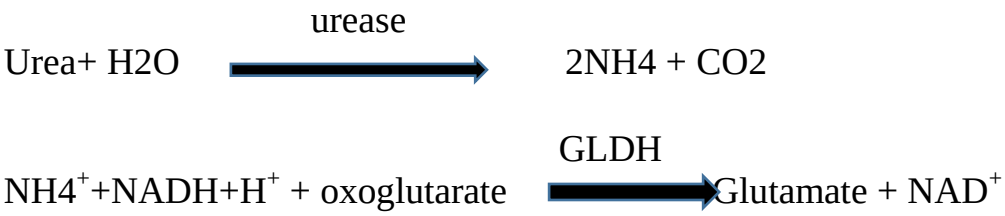
**ESTIMATION OF BLOOD UREA**

**KIT** : Accucare

**Method** : UV - GLDH

**Principle :** The test is performed as a kinetic assay in which the initial rate of the reaction is linear for a limited period of time. Urea in the sample is hydrolysed by urease to $NH_3$ and $CO_2$. The $NH_3$ produced

combines with alpha-oxoglutarate and NADH in the occurrence of glutamate dehydrogenase to produce glutamate and NAD.




The initial rate of decrease in absorbance at 340nm is proportional to the urea concentration in the sample.

**Reagent**

Reagent I: buffer reagent

Reagent II: enzyme reagent

Urea standard: 50 mg/dl

Mix 4 parts (4 ml) of buffer reagent with one part (1 ml) of enzyme reagent

**Procedure**

To 1 ml of the reconstituted reagent 10µl of serum is added andimmediately reading was taken at 340 nm.

**Reference Range**

Serum/ plasma : 15- 40 mg/dl

## ESTIMATION OF SERUM CREATININE

**Kit used** : ERBA

**METHOD** : Jaffe's Method ,Initial rate

**Principle** : Creatinine in alkaline solution reacts with picrate to form a orange-yellow compound. The colour is proportional to the concentration of creatinine in the sample when measured at 505nm.

**Reagent**

Reagent I : Picric acid

Reagent II : Sodium hydroxide

Creatinine standard: 2 mg/dl

Reagents were allowed to attain room temperature. Equal volumes of reagent 1 and reagent 2 were mixed , waited for 15 minutes before use .

**Procedure**

To 1 ml of the reconstituted reagent 100µl of the serum were added and absorbance (A1 ) at 20 seconds after mixing was noted & final absorbance ((A2 ) at 80 seconds .

**Calculation**

A = A2 - A1

$$\text{Creatinine (mg/dl)} = \frac{\text{Absorbance of Test X concentration of standard ( mg/dl)}}{\text{Absorbance of standard}}$$

**Reference Values**

Males : 0.9-1.3mg/dl

Females : 0.6-1.1mg/dl

## ESTIMATION OF SERUM TRIGLYCERIDE

**Method** : Enzymatic colorimetric method : GPO – PAP method

**Kit used** : ERBA

**Principle** :

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-3-Phosphate} + ADP$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{Glycerol-3-Phosphate Oxidase}} \text{Dihydroxyacetone Phosphate} + H_2O_2$$

$$2\,H_2O_2 + 4\text{Aminoantipyrine} + \text{DHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + H_2O$$

DHBS- 3,5-Dichloro- 2 hydroxy benzene sulfonate

The intensity of colour developed during the reaction is proportional to the triglyceride level in the sample and is measured at 505nm.

**Procedure**

To 1 ml of the reconstituted reagent 10µl of serum & 10µl of standard added separately and read at 546 nm after incubation at 37°c for10 min.

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test X concentration of standard ( mg/dl)}}{\text{Absorbance of standard}}$$

**Reference range**

Normal fasting levels - < 150 mg/dl

**ESTIMATION OF SERUM TOTAL CHOLESTEROL**

**Method** : CHOD - PAP method

**Kit used** : ERBA

**Principle** :

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneiminedye} + 4H_2O$$

The concentration of cholesterol is proportional to the intensity of the red complex, which is measured at 505 nm.

**Procedure**

To 1ml of the reconstituted reagent, 10µl of serum & 10µl of standard was added separately and reading was taken after 5 minutes of incubation at 37°c.

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of Test X concentration of standard ( mg/dl)}}{\text{Absorbance of standard}}$$

**Reference values**

Cholesterol: 150 to 200 mg/dl

**ESTIMATION OF HDL CHOLESTEROL**

**Method** : Direct enzymatic clearance assay

**Kit used** : Spinreact

Enzyme Reagent 1

N,N bis(2-hydroxyethyl)-2-aminoethanesulphonic acid 100 millimoles , pH 6.6(25°C)

2 Aminoethanosulfonic acid

N-(2- hydroxyl-3- sulfopropyl)-3-5 dimethoxyaniline (HDAOS)

Cholesterol esterase

Cholesterol oxidase

Catalase

Ascorbic oxidase

Enzyme Reagent 2

N,N bis(2-hydroxyethyl)-2-aminoethanesulphonic acid 100 mM ,pH 6.6(25°C)

2 Aminoethanosulfonic acid , 4- Aminoantipyrine

Peroxidase, sodium azide ,surfactants

**Principle**

Two steps

**1. Elimination of chylomicron,VLDL- cholesterrol,LDL cholesterol :**

Cholesterol esterase

Cholesterol Esters ⟶ Cholesterol+Fatty acids

Cholesterol Oxidase

Cholesterol + O2 ⟶ 4-Cholestenone + H2O2

catalase

2 H2O2 ⟶ $H_2O + O_2$

**2. Specific measurement of HDL cholesterol:**

Intensity of colour formed is proportional to HDLc concentration in the sample

Cholesterol esterase

Cholesterol Esters ⟶ Cholesterol+Fatty acids

Cholesterol Oxidase

Cholesterol + O2 ⟶ 4-Cholestenone + H2O2

Peroxidase

2 H2O2 + HDAOS+ 4-AAP ⟶ quinonmine +$4H_2O$

**Procedure**

Blank - 300 µ l of reagent 1 was taken

Test - To 300µl of reagent 1 add 5 µl of serum was added and incubation done at 37 °C for five minutes.

Standard/Randox Controls - To 300µl of reagent 1 add 5 µl of calibrator was added and incubation done at 37°C for five minutes

Absorbance (A1) of the samples & calibrator at 600 nm was noted, 100µl of reagent 2 into blank, calibrator & sample was added

Mixed and incubation done for 30 sec.

Final absorbance (A2) of sample, calibrator, against the blank was noted

Incubate for 150 sec , Read final absorbance (A2)

Calculate the increase of the absorbance A = A2 - A1

$$\text{Calculations} = \frac{\text{Absorbance of sample x calibrator concentration}}{\text{Absorbance of calibrator}}$$

**Reference value**

| | **Men** | **Women** |
|---|---|---|
| Normal | 35 – 50 mg/dl | 45 – 60 mg/dl |
| Low risk | > 50 mg/dl | >60 mg/dl |
| High risk | < 35 mg/dl | < 45 mg/dl |

## ESTIMATION OF LDL CHOLESTEROL

**Method** : Direct enzymatic colorimetric

**Kit used** : Spinreact

### Reagent 1

PIPES pH 7.0, 50 millimoles/L

Piperazine – 1,4 bis(2-Ethanosulfonic acid)
TOOS

N- ethyl-N- (2- hydroxyl-3- sulfopropyl)-3- methyl aniline
Cholesterol esterase(CHE)
Cholesterol oxidase (CHOD)

Catalase

### Reagent 2

PIPES Buffer 50 pH 7.0

Piperazine – 1,4 bis(2-Ethanosulfonic acid)

4- Aminoantipyrine

Peroxidase (POD)

Sodium azide

### Principle

Two steps

**1. Elimination of chylomicron,VLDL- cholesterrol, HDL cholesterol**

$$\text{Cholesterol Esters} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol+Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{4-Cholestenone} + H_2O_2$$

$$2\,H_2O_2 \xrightarrow{\text{catalase}} H_2O + O_2$$

**2. Specific measurement of LDL cholesterol:**

Intensity of color formed is proportional to LDLc concentration in the sample

Cholesterol esterase

Cholesterol Esters ⟶ Cholesterol+Fatty acids

Cholesterol Oxidase

Cholesterol + O2 ⟶ 4-Cholestenone + H2O2

Peroxidase

2 H2O2 + HDAOS+ 4-AAP ⟶ quinonimine +4H $_2$O

Blank - Take 225 µl of reagent 1
Sample - To 225 µl of reagent 1 add 3 µl of serum was added and incubate for 5 min at 37° C.
Standard - To 225 µl of reagent 1 add 3 µl of standard was added and incubate for 5 min at 37°c.
Initial absorbance A1 was noted.
Add 75 µl of reagent 2 into blank, standard & sample

Incubation done at 37°C for five minutes.

Read the final absorbance (A2 ) of sample, standard, against the blank at 600nm

$$\text{LDL concentration mg Dl} = \frac{\text{Absorbance of sample x Absorbance of standard}}{\text{Absorbance of standard}}$$

**Reference value**

Optimal - less than100 mg/dl

Near optimal - 100- 129 mg/dl

Borderline high - 130- 160 mg/dl

High - more than160 mg/dl

## ESTIMATION OF APOLIPOPROTEIN B

**Method** : Turbidimetry

**Kit Used** : Spinreact

**Reagent** :

Diluent (R1) –Tris buffer 20 mmol/L, PEG, pH 8.3 & sodium azide 0.95 g/dl

Antibody (R2) – goat serum, anti-human Apo B, tris 50 mmol/L,

pH 7.5, sodium azide 0.95 g/dl

Apo B cal- 85 mg/dl.

**Principle**

Turbidimetry

Insoluble complexes formed by samples containing apo B mixed with anti-apoB antibodies. The change in absorbance caused by these complexes proportional to apo B levels in serum sample.

**Procedure**

1. Bring the reagents & photometer to 37°C.

2. Assay conditions

Wave length: 340 nm

Temperature: 370C

Cuvette light path: 1 cm

3 . The instrument was adjusted to zero with distilled water

4 . Pipette in to a cuvette

Reagent R1 268 µl

Sample or calibrator 2.3µl

5 . Mixed & the absorbance (A1) after sample addition is noted.

6 . 67 µl of reagent 2 to calibrator & sample was added, mixed & absorbance (A2) after two minutes after addition of R2 was taken.

**Calculations**

Calculate the A2 – A1 of each point of the calibration curve and the values against the Apo B concentration of each calibrator was plotted. Apo B concentration in the sample was calculated by interpolation of its (A2 – A1) in the curve.

**Reference range**

Normal value – 69 – 105 mg/dl

**ESTIMATION OF APOLIPOPROTEIN A-1**

**Method** : Turbidimetry

**Kit Used** : Spinreact

**Reagent** :

Diluent(R1) - Tris buffer 20 mmol/L,PEG, pH8.3 & sodiumazide 0.95 g/dl

Antibody (R2) – goat serum, anti-human Apo A-1, tris 50 mmol/L,

pH 7.5, sodium azide 0.95 g/dl

Apo A1 cal- 108 mg/dl.

**Principle**

Turbidimetry

Insoluble complexes formed by samples containing apo A1 mixed with anti-apoA1 antibodies.the change in absorbance caused by these complexes proportional to apo A1 levels in serum sample.

**Procedure**

1. Bring the reagents & photometer to 37 °c.

2. Assay conditions

Wave length: 340 nm

Temperature: 37°c

Cuvette light path: 1 cm

3. Adjust the instrument to zero with distilled water

4. Pipette in to a cuvette

Reagent R1 268 µl

Sample or calibrator 2.3 µl

5. Mix & read the absorbance (A1) after sample addition

6. 67 µl of reagent 2 to calibrator & sample was added, mixed & absorbance (A2) exactly 2 minutes after addition of R2 was taken.

**Calculations**

A2 – A1 of each point of the calibration curve was calculated and plotted the values against the Apo A-1 concentration of each calibrator dilution. Apo A-1 concentration in the sample is calculated by interpolation of its (A2 – A1) in the curve.

**Reference range**

Normal value : 122 – 161 mg/dl

I. For gene polymorphism studies fasting blood was collected in2 ml EDTA coated tube.

## DNA EXTRACTION BY KIT METHOD:

## DNA MINIPREPARATION KIT:

From helini biomolecules, Chennai.

## PRINCIPLE :

On short incubation with proteinase K and in the presence of chaotropic salt, cells are lysed which immediately inactivates all nucleases. Nucleic acids in the cells , attach selectively to special glass fibres pre-packed in the Purefast purification filter tube. In a series of rapid 'wash and spin' steps it removes the contaminating cellular components ,thus bound nuclei acids get purified. A special inhibitor removal buffer was added , this allows even the application of heparinised sample material with 100U/ml of heparin. Finally low salt elution buffer releases the nucleic acids from the glass fibre. This method eliminates the need for organic solvent extractions and DNA precipitation, allowing for rapid purification of many samples simultaneously.

## COMPONENTS OF THE KIT

- Proteinase K solution
- Lysis buffer

- Wash buffer 1 & 2
- Isopropranolol
- Elution buffer
- Spin columns with collection tubes

**PROTEINASE K SOLUTION STORAGE:**

Solution were stored at -20 °. It is stable for at -20°C for upto 6 months.

**PROCEDURE FOR DNA PURIFICATION:**

**PRE-PROCEDURE STEPS:**

- Set water bath to 56°C
- Warm elution buffer by keeping in water bath at 56°C
- Prepare fresh 1.2 ml of 70% ethanol per sample.

**BLOOD / BUFFYCOAT:**

200 µl of blood or 200µl of buffy coat is added to a nuclease free 1.5ml microcentrifuge tube and the following steps are carried out.

1. 400µl of lysis buffer is added and
2. Immediately mixed well by inverting several times (or gently vortex)

3. 20µl of proteinase K was added.

4. Mixed by vortex for 10 seconds.

5. 300µl of isopropranolol was added and mixed well by inverting several times.

6. The sample was pipetted into the Purefast spin column and

7. Centrifuged at 12,000rpm for 1min.

8. The flow-through was discarded and the column is positioned back into the same collection tube.

9. 500µl of Wash buffer I was added to the Purefast spin column and

10. Centrifugation process for 1minute at 12,000rpm ,and the flow-through is discarded and the column was placed back into the same collection tube.

11. 500µl wash buffer II was added to the Purefast spin column

12. Centrifuged at 12,000rpm for one minute and the flow-through discarded the column was placed back into the same collection tube.

13. Do empty spin column centrifuge at 13000rpm for 2 minutes.

14. The flow-through was discarded and centrifuged for an additional. This step was essential to avoid residual ethanol.

15. The Purefast spin column is transferred into a fresh 1.5 ml microcentrifuge tube.

16. 60µl of the pre-warmed elution buffer was added to the centre of Purefast spin column membrane.

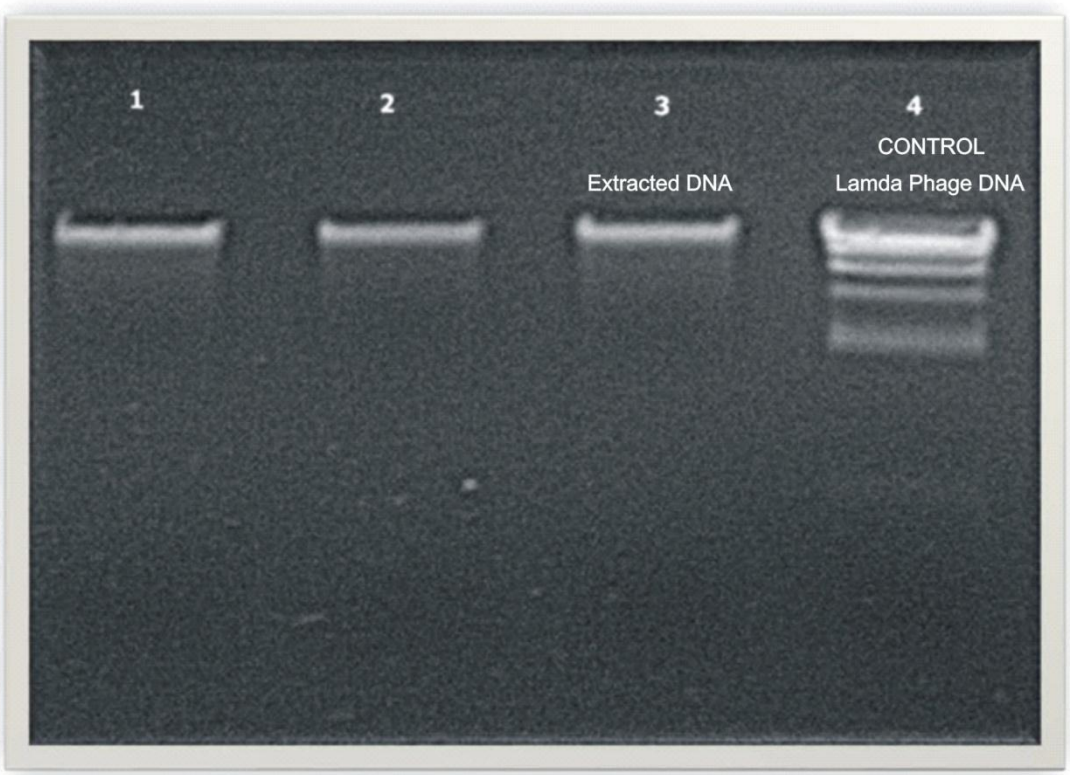
17. Take care not to contact the membrane with the pipette tip. Incubation pre warmed was done at room temperature for two minutes and is centrifugedfor two minutes.

18. The column was discarded and the purified DNA, was deep freezed at - 20° C.

**IDENTIFICATION**

Extracted DNA was identified by 1% agarose gel electrophoresis and comparison with a known molecular weight 1kb DNA (Lambda DNA) ladder .(Fig.15)

**FIG.15 EXTRACTED DNA**



## POLYMERASE CHAIN REACTION

### C+83T ( First Intron ):

353 bp fragment of Apo A1gene was amplified using,

- Forward primer:

  5’GGCCACGGGGATTTAGGGAGAA-3’

- Reverse primer:

  5’AGCTGGCTGCTTAGAGACTGCGA-3’

### G–75A (Promoter region):

203bp fragment of Apo A1gene was amplified using,

- Forward primer :

  5’-GCAGCTTGCTGTTTGCCCACTC-3’

- Reverse primer :

  5’-ACGCACCTCCTTCTCGCAGTCT-3’

  (Primers for both the sites were designed by helini biomolecules)

## PRIMER RECONSTITUTION

Primers were supplied in lyophilized form. Millipore double distilled water was used to prepare 100× concentrations i.e. 10 times the molecular weight of primer is the volume of water required to prepare 100× concentrations which is 100µmolar solution.

From this stock solution 10× concentration was prepared as the working solution for PCR.

Both forward and reverse primer were spinned.

For each polymorphic site TEA buffer was taken at a particular volume corresponding to the respective forward & reverse primer of 100 pmol/µl .

Inverted & Vortexed &mixed for 5 minutes & were centrifuged

Eppendorf were named for both the polymorphic sites.

180 µl of sterile distilled water were taken in the labelled Eppendorf.

10 µl forward primer + 10 µl reverse primer were added

200 µl of reconstituted primers containing both forward &reverse primers for the respective polymorphic sites.

**MASTER MIX:**

2×PCR Master mix was used in the following composition

Master Mix consists of basic components necessary for PCR.

Reaction buffer consisted of Tris Hcl - pH 8.5 , $(NH_4)_2SO_4$ ,MgCl2 – 3 mM acts as catalyst &0.2%Tween 20

dNTP’ s were used in a concentration of 0.4 mM each.

Taq polymerase in a concentration of 0.2 U/ µl

Primers were used in a concentration of 10 pmol and DNA was used in a concentration of 200ng.

PCR was carried out in a reaction in volume of 20µL with the following components in the following manner,

| | |
|---|---|
| PCR master mix (contains gel loading dye) | – 10 µL |
| Reconstituted primers(for the site+83 ) | – 5.0 µL |
| DNA | – 5.0µL |
| Total | - 20.0µL |

**POLYMERASE CHAIN REACTION (PCR)**

Amplification of the extracted DNA was carried out in CYBERLAB SMART PCR-PRO, thermal cycler with the following cycling conditions

**STEPS IN PCR C+83T (first intron) POLYMORPHIC SITE:**

- Initial denaturation - 95° C /5min

  34cycles of

- Cycle Denaturation at 94°C for 30 seconds
- Cycle Annealing at 58°C for 30 seconds
- Cycle Extension at 72°C for 30 seconds
- Final Extension at 72°C for 10min

Amplified product – amplicons of 353 bp was identified by 2.5% agarose gel electrophoresis by comparison with a known 100bp DNA ladder.

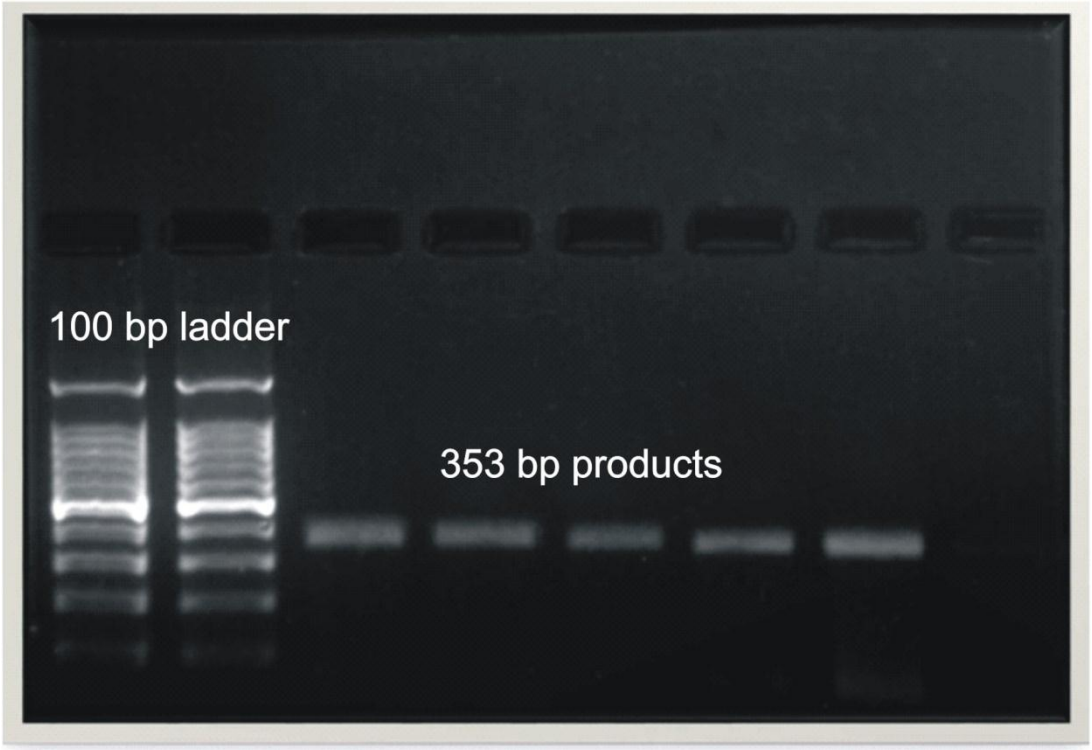
## AGAROSE GEL ELECTROPHORESIS

- PCR product was run on 2.5% agarose gel in a 25 mL agarose cast as follows: 0.625g of agarose was weighed and dissolved in 25 mL of TAE buffer with a pH of 8.0.

- It was microwaved for 60 secs, cooled and ethidium bromide was added in a concentration of 0.5 µg/ml from the stock of 10mg/mL added. It is poured into a cast and allowed to solidify for 45 min before it wss kept in the electrophoresis tank.

- 10µL of PCR product was loaded onto wells and 4µL of 100bp DNA ladder was loaded onto single well as a marker. It is run in an electrophoresis tank for 30min and visualized under UV illumination in e gel imager from life technologies.(Fig.16)

**FIG.16 PCR PRODUCT FOR C+83T (FIRST INTRON)**



## RESTRICTION FRAGMENT LENGTH POLYMORPHISM

Apo A1 gene polymorphism at the site C+83T was spotted by digestion of the amplified PCR product with the MspI restriction enzyme trailed by run in 2.5% agarose gel electrophoresis.

## Restriction Digestion Procedure

| | |
|---|---|
| Tango buffer (1x) | – 3 µL for 100%MspI digestion |
| Msp I (10U/µL) | – 2.0 µL |
| PCR Product | – 10.0 µL |
| Distilled water | – 15.0 µL |
| Total | 30.0 µL |

- The entire procedure was carried out in ice. The contents were mixed thoroughly.
- The eppendorf was then placed in a 37°C water bath for 1 hour .
- Restriction digested product was subjected to 2.5% agarose gel electrophoresis for genotyping.

Two restriction sites for Msp I with in 353 bp product.

## RESTRICTION DIGESTED PRODUCT VIEWED BY RUNNING IN 2.5% AGAROSE GEL ELECTROPHORESIS

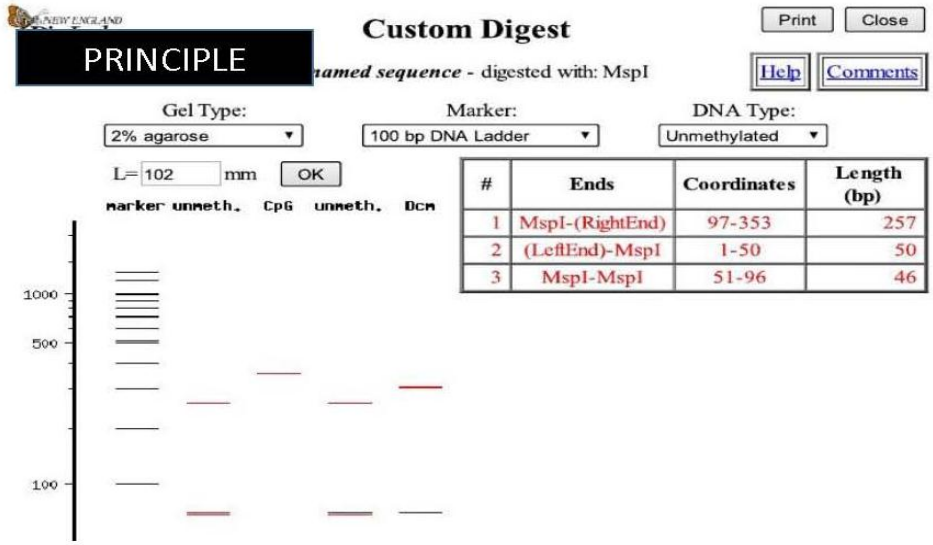
## PROCEDURE:

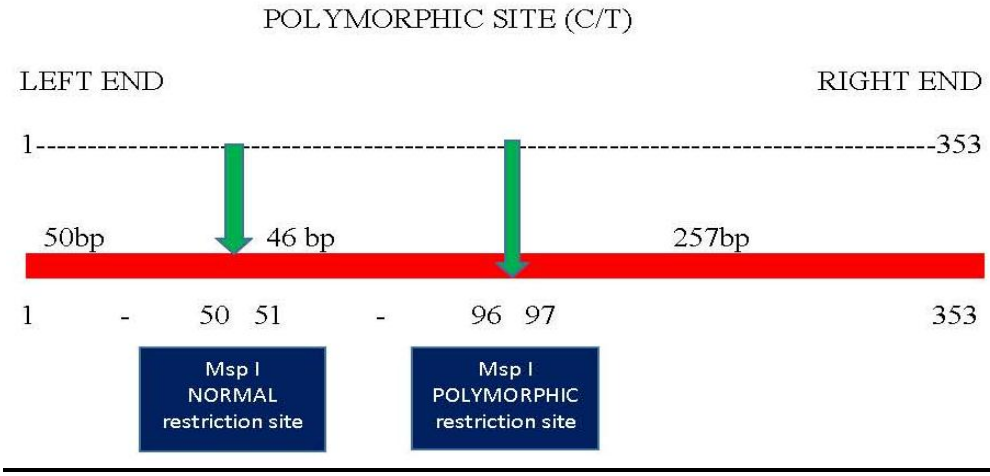
- Restriction Digestion product was run on 2.5% agarose gel in a 25mL agarose cast as follows: 0.625g of agarose was weighed and dissolved in 25mL of TAE buffer with a pH of 8.0.

- It was microwaved for 60 secs, cooled and Ethidium bromide concentration 0.5 µg/ml from the stock of 10mg/mL was added. It was poured into a cast and allowed to solidify for 45 min before it was kept in the electrophoresis tank.

- 20µL of Restriction Digestion product was loaded onto wells and 4µL of 100bp DNA ladder was loaded onto single well as a marker. It was run in an electrophoresis tank for 45min and visualized under UV illumination in e-gel imager from life technologies. (Fig.17)

Analysis was done using 100 bp and 50 bp ladder.



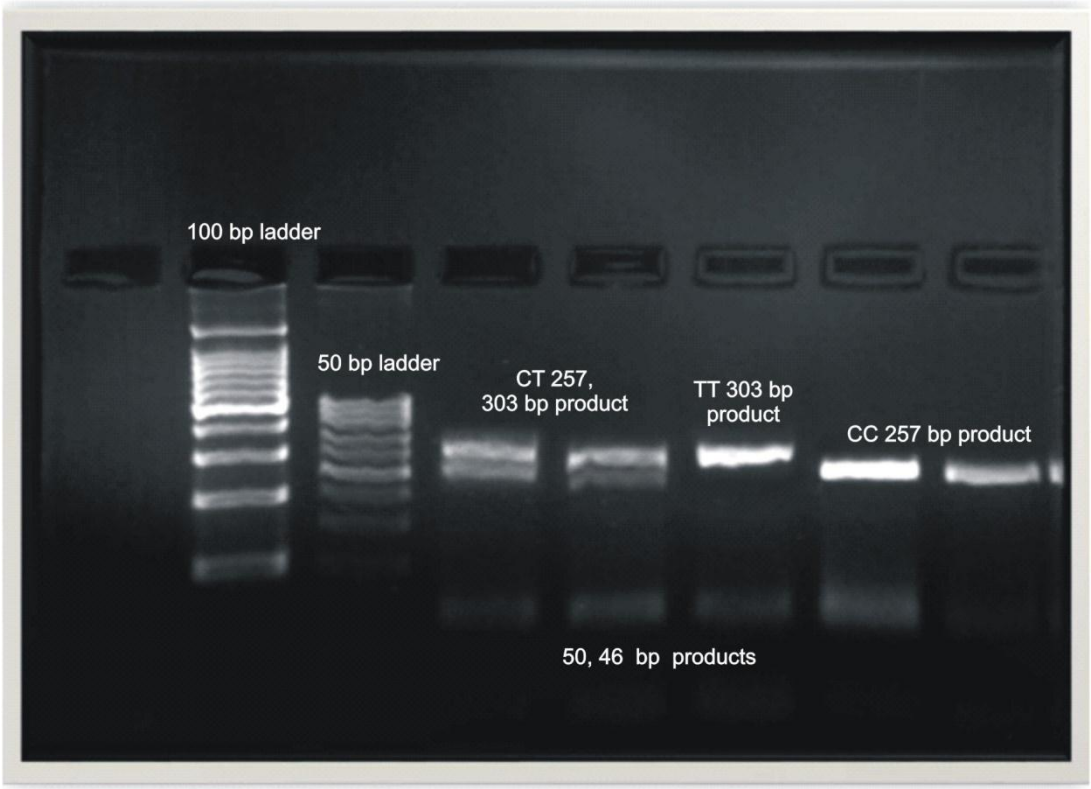




**C ALLELE-** have restriction site between 96-97 bp ( polymorphic site) as well as normal restriction site at 50-51bp .

**T ALLELE**- destroy its site for restriction at position 96-97bp

- **CC (homozygous individuals)**- will yield 257bp,46bp,50bp
- **CT (heterozygous individuals)**- will yield 257bp, 46bp, 50bp, 303bp, 50bp
- **TT(homozygous individuals)**- will yield 303bp,50bp

**FIG.17 <u>RESTRICTION DIGESTION FRAGMENTS C+83T (FIRST INTRON)</u>**



## G-75A POLYMORPHISM(PROMOTER REGION)

### I.EXTRACTED DNA SUBJECTED TO PCR AMPLIFICATION

### POLYMERASE CHAIN REACTION

PCR was carried out in a reaction in volume of 20µL with the following components in the following manner,

| | |
|---|---|
| PCR master mix (contains gel loading dye) | – 10 µL |
| Reconstituted primers (for the site -75) | – 5.0 µL |
| DNA | – 5.0 µL |
| Total | 20.0 µL |

Amplification of the extracted DNA was carried out in CYBERLAB SMART PCR-PRO, thermal cycler with the following cycling conditions

### STEPS IN PCR FOR THE G-75 A(PROMOTER REGION) POLYMORPHIC SITE:

- Initial denaturation - 95°C /5min

  34cycles of

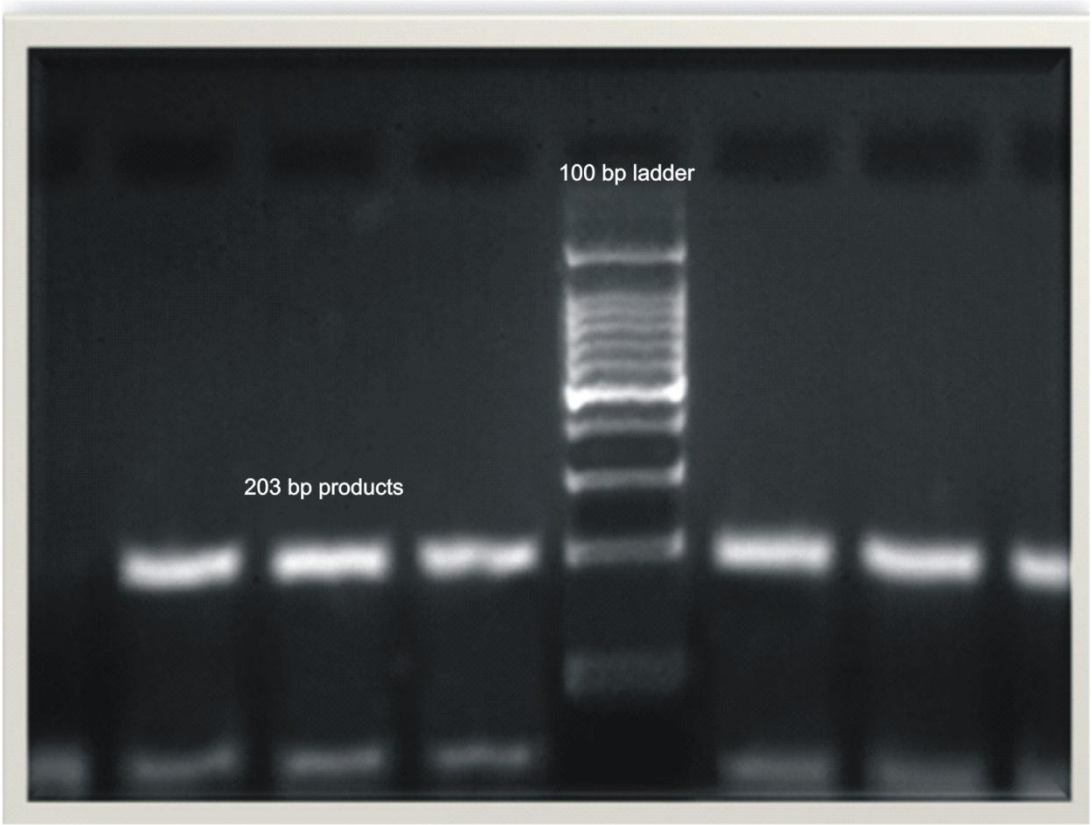
- Cycle Denaturation at 94°C for 30 sec
- Cycle Annealing at 55.9°C for 30 sec
- Cycle Extension at 72°C for 30 sec
- Final Extension at 72° C for 10min

Amplified product – amplicons of 203 bp was identified by 2.5% agarose gel electrophoresis by comparison with a known 100bp DNA ladder.

## AGAROSE GEL ELECTROPHORESIS

- PCR product was run on 2.5% agarose gel in a 25 mL agarose cast as follows: 0.625g of agarose was weighed and dissolved in 25 mL of TAE buffer with a pH of 8.0.
- It was microwaved for 60 secs, cooled and ethidium bromide was added concentration 0.5 µg/ml from the stock of 10mg/mL was added. It was poured into a cast and allowed to solidify for 45 min before it was kept in the electrophoresis tank.
- 10µL of PCR product was loaded onto wells and 4µL of 100bp DNA ladder was loaded onto single well as a marker. It was run in electrophoresis tank for 30min and visualized under UV illumination.(Fig.18)

**FIG.18 PCR PRODUCT FOR G – 75 A (PROMOTER REGION)**



## RESTRICTION DIGESTION OF THE PCR PRODUCTS

## RESTRICTION FRAGMENT LENGTH POLYMORPHISM

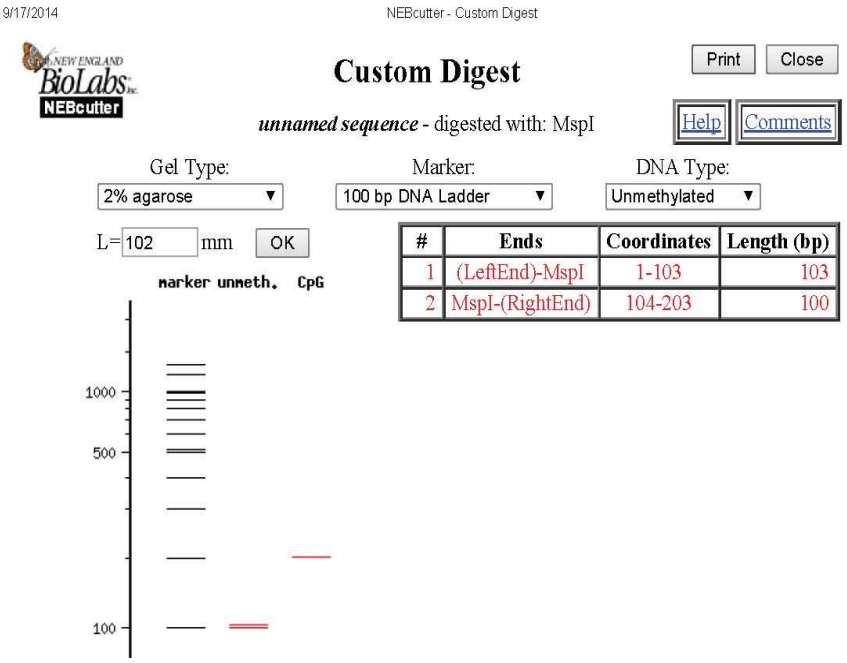
Apo A1 gene polymorphism at the site **G–75A (PROMOTER REGION)** was spotted by digestion of the amplified PCR product with the MspI restriction enzyme trailed by run in 2.5% agarose gel electrophoresis.

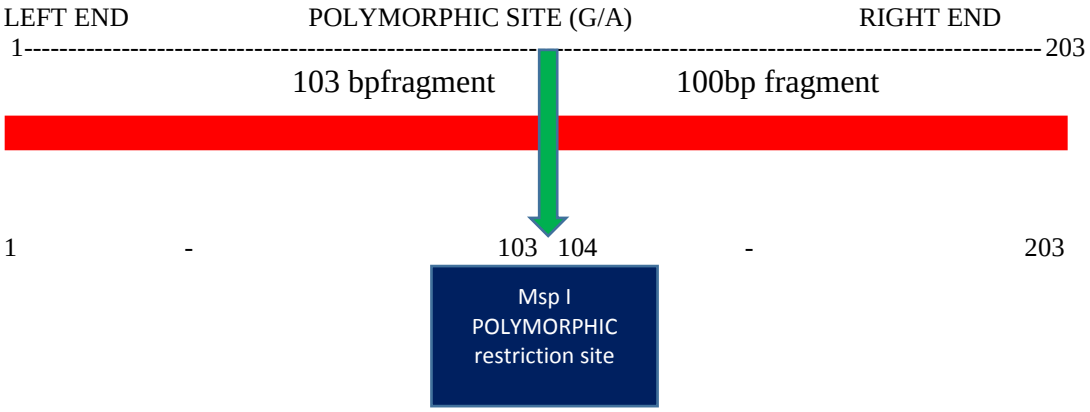
### Restriction Digestion Procedure

| | |
|---|---|
| Tango buffer (1x) | – 3 µL for 100%MspI digestion |
| Msp I (10U/ µL) | – 2.0 µL |
| PCR Product | – 10.0 µL |
| Distilled water | – 15.0 µL |
| Total | 30.0 µL |

- The entire procedure was carried out in ice. The contents were mixed thoroughly.
- The eppendorf was then placed in a 37°C waterbath for 1 hour .

Restriction digested product was subjected to 2.5% agarose gel electrophoresis for genotyping.(Fig.19)



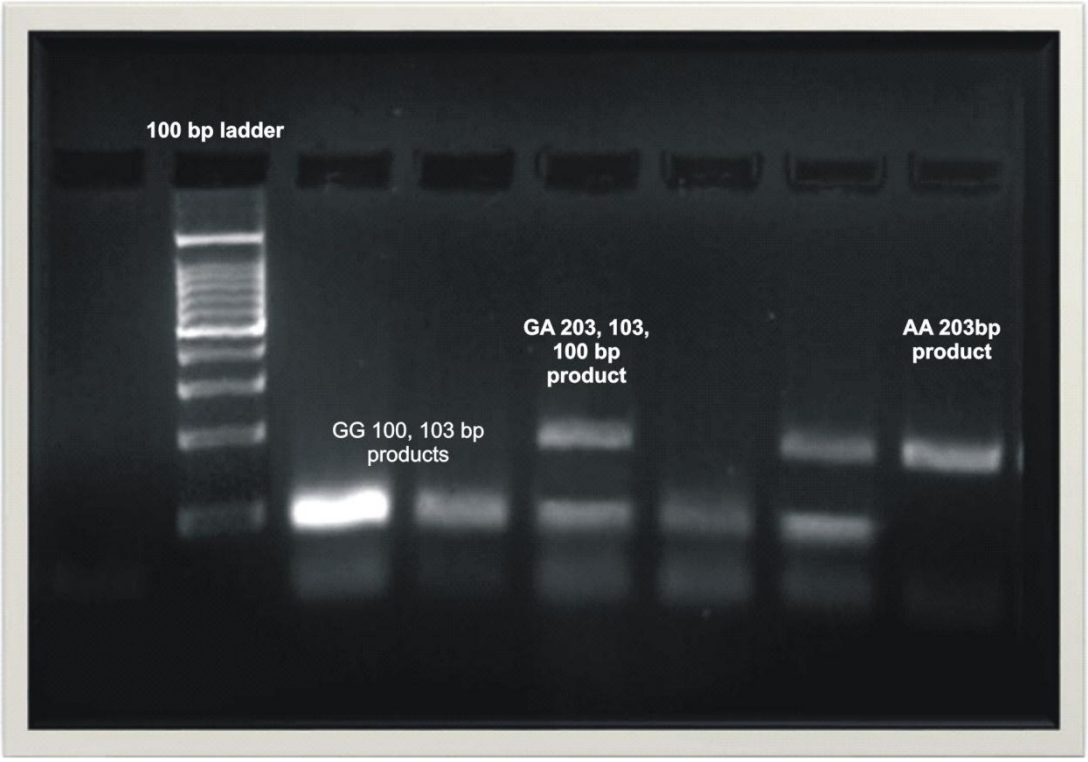

One restriction site for Msp I within 203 bp

**G ALLELE-** have restriction site between 103-104 bp (polymorphic site) yielding 103bp &100bp

**A ALLELE**- destroy its site for restriction at position 103-104bp , so

- **GG (homozygous individuals)**- will yield 103 & 100 bp
- **GA (heterozygous individuals)**- will yield 203bp,103bp &100bp
- **AA (homozygous individuals)**- will yield only 203bp

**FIG.19 RESTRICTION DIGESTION FRAGMENTS OF G–75A (PROMOTER REGION)**



# DATA COLLECTION

## PROCEDURES ANDINSTRUMENTS USED

Data collection was done using standardized proforma. All the biochemical analyses were performed using automated (ROBONIK) clinical chemistry analyzer .

## QUALITY CONTROL

All biochemical analyses were done using RANDOX calibrator (lot no 2351-562UE) and Controls (lot no 768UN level 2) for checking internal quality. The CVs of all the analytes performed were within the prescribed limits in accordance with CLIA.

## INSTRUMENTS USED FOR MOLECULAR ANALYSIS :

1. Cooling microcentrifuge
2. Millipore double distilled water
3. Waterbath
4. Ice flaker
5. Minifuge
6. PCR machine
7. Electrophoresis apparatus
8. Gel Doc

## CONFIDENTIALITY

Explicit informed consent was acquired from all patients. Confidentiality and safety of the subjects were taken care.

# RESULTS

**Master table1**: gives the characteristics of MI patients along with their biochemical parameters Fasting Blood Glucose ,Urea , Creatinine, Total cholesterol(TC),TGL,HDL,LDL,ApoA1,ApoB,Genotype G-75A,C+83T

**Master table 2:** gives the characteristics of control group along with their biochemical Parameters, Fasting blood sugar ,urea , creatinine, Total cholesterol,Triglyceride,HDL,LDL,Apo A1,Apo B,Genotype G-75A,C+83T

The mean for each parameter is also given in the table.

## STATISTICAL ANALYSIS

1) SPSS package was used for statistical analysis

   a) Pearson's correlation was used for univariate analysis.

   b) The groups were compared using Student's t test.

   c) p value of $<0.05$ is considered significant

2) Genotype frequency distribution between cases and controls werecompared with the $x^2$ test for 2×2 contingency table.

3) Analysis of variance (ANOVA) was used to know the relationship between fasting serum HDL, Apo B ,Apo A1,ratio of Apo B/Apo A1with the genotype distribution.

52 Acute Myocardial patients and 52 Age, Gender ,Risk Factor matched controls were analysed for genotype distribution of **APOA1 GENE POLYMORPHISMG–75A (PROMOTER REGION) AND C+83T (FIRST INTRON)**.The genotype distribution was studied with respect to as fasting serum levels of HDL, Apo B , Apo A1,Apo B /Apo A1 ratio.

## TABLE:1

## BASELINE CHARACTERISTICS OF THE TWO GROUPS

| VARIABLE | GROUP TOTAL | NO OF PERSONS | | p VALUE |
|---|---|---|---|---|
| **AGE** | CASES(52) | 53.77±8.34 | | 0.92 (NS) |
| | CONTROLS(52) | 53.6 ±10.04 | | |
| **GENDER DISTRIBUTION** | CASES(52) | MALE | 45 | 1 (NS) |
| | | FEMALE | 7 | |
| | CONTROLS(52) | MALE | 44 | |
| | | FEMALE | 8 | |
| **H/O SMOKING** | CASES(52) | YES | 16 | 0.257 (NS) |
| | CONTROLS(52) | YES | 10 | |
| **H/O ALOCOHOL** | CASES | YES | 17 | 0.669 (NS) |
| | CONTROLS | YES | 14 | |
| **H/O CAD** | CASES(52) | YES | 8 | 0.003 (S) |
| | CONTROLS(52) | YES | 0 | |
| **DM** | CASES(52) | YES | 24 | 0.692 (NS) |
| | CONTROLS(52) | YES | 21 | |
| **HT** | CASES(52) | YES | 13 | 0.816 (NS) |
| | CONTROLS(52) | YES | 11 | |

- The mean total Age value of cases is 53.77 and controls is 53.60 p=0.92(NS)

- The GENDER DISTRIBUTION in cases and controls were, 45 males and 7 females in cases and 44 males and 8 females in controls, $X^2$ - 0.078 , p= 1.0 (NS)

- The number of SMOKERS in cases were 16 and in controls -10 , $X^2$ - 1.846, p= 0.257 (NS)

- The number of ALCOHOLICS in cases were 17 and in controls -14 , $X^2$ - 0.414 , p= 0.669(NS)

- The number of persons with H/O CAD in cases were 8 and in controls -nil , $X^2$ - 8.667 , p= 0.006 (S)

- The number of persons with H/O DM in cases were 24 and in controls -21 , $X^2$ - 0.353, p= 0.692(NS)

- The number of persons with H/O HT in cases were 13 and in controls -11 , $X^2$ - 0.217, p= 0.816(NS)

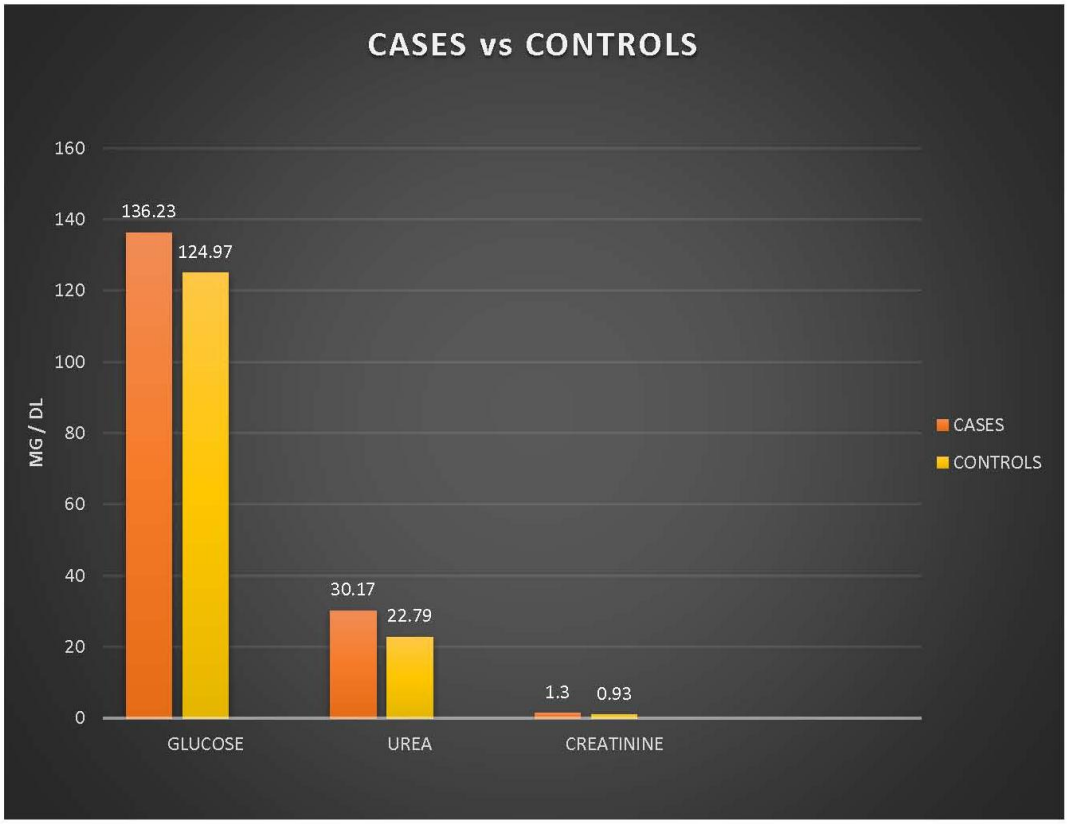
## TABLE 2

## COMPARISON OF AGE , BLOOD SUGAR ,UREA & CREATININE BETWEENCASES AND CONTROLS

| VARIABLE | GROUP | TOTAL | MEAN | SD | p VALUE |
|---|---|---|---|---|---|
| GLUCOSE | CASES | 52 | 136.23 | 76.63 | 0.43(NS) |
| | CONTROLS | 52 | 124.94 | 70.58 | |
| UREA | CASES | 52 | 30.17 | 26.20 | 0.05(NS) |
| | CONTROLS | 52 | 22.79 | 7.30 | |
| CREATININE | CASES | 52 | 1.30 | 1.05 | 0.01(S) |
| | CONTROLS | 52 | 0.93 | 0.15 | |

- The mean GLUCOSE value of cases is 136.23 mg/dl and controls is 124.94 mg/dl, p= 0.43.
- The mean UREA value of cases is 30.17mg/dl and controls is 22.79mg/dl, p=0.05.
- The mean CREATININE value of cases is 1.30 mg/dl and controls is 0.93; p-0.01

**BAR DIAGRAM SHOWINGCOMPARISON BETWEEN CASES AND CONTROLS ON FASTING SERUM GLUCOSE ,UREA, CREATININE**



## TABLE 3

## COMPARISON OF SERUM TOTAL HOLESTEROL, TRIGLYCERIDE AND LDL LEVELS BETWEEN CASES AND CONTROLS

| VARIABLE | GROUP | TOTAL | MEAN | SD | p VALUE |
|---|---|---|---|---|---|
| TOTAL CHOLESTEROL | CASES | 52 | 197.42 | 57.05 | 0.005(S) |
| | CONTROLS | 52 | 169.33 | 42.40 | |
| TRIGLYCERIDE | CASES | 52 | 164.13 | 60.85 | 0.001(S) |
| | CONTROLS | 52 | 128.90 | 44.52 | |
| LDL | CASES | 52 | 118.52 | 43.50 | 0.03(S) |
| | CONTROLS | 52 | 101.44 | 39.06 | |

- The mean TOTAL CHOLESTEROL value of cases is 197.42mg/dl and controls is 169.33 mg/dl, p=0.005.
- The mean TRIGLYCERIDE value of cases is 164.13 mg/dl and controls is 128.9 mg/dl, p= 0.001.
- The mean LDL value of cases is 118.52mg/dl and controls is 101.44mg/dl, p=0.03

# BAR DIAGRAM SHOWING COMPARISON BETWEEN CASES AND CONTROLS ON SERUM TOTALCHOLESTEROL, TRIGLYCERIDE & LDL LEVELS

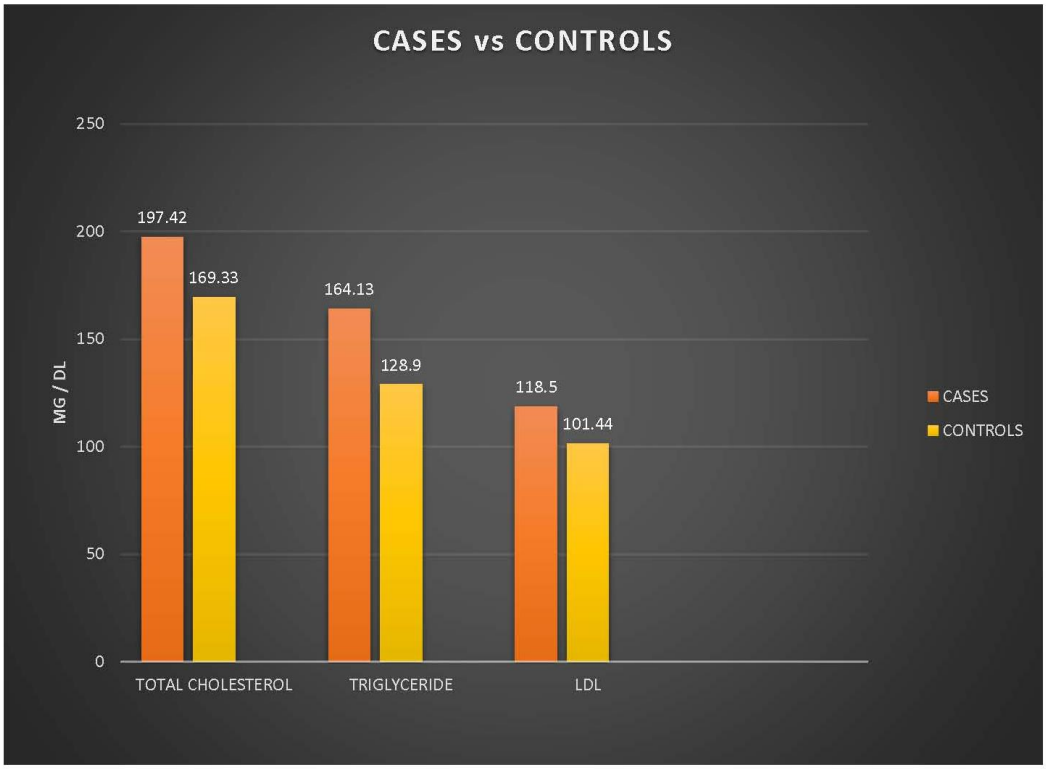


**TABLE 4**

**COMPARISON OF SERUM HDL, APO A1,APO B ,APOB/APO A RATIO BETWEEN CASES AND CONTROLS**

| VARIABLE | GROUP | TOTAL | MEAN | SD | p VALUE |
|---|---|---|---|---|---|
| HDL | CASES | 52 | 48.54 | 12.74 | 0.000 (S) |
| | CONTROLS | 52 | 63.08 | 13.80 | |
| APO A1 | CASES | 52 | 81.46 | 22.30 | 0.000 (S) |
| | CONTROLS | 52 | 114.02 | 18.14 | |
| APO B | CASES | 52 | 98.79 | 25.04 | 0.004 (S) |
| | CONTROLS | 52 | 84.48 | 23.84 | |
| APO B /APO A | CASES | 52 | 1.29 | 0.52 | 0.000(S) |
| | CONTROLS | 52 | 0.74 | 0.21 | |

- The mean HDL value of cases is 48.54 mg/dl and controls is63.08 mg/dl, p=0.000.
- The mean APO A1 value of cases is 81.46 mg/dl and controls is 114.02mg/dl, p= 0.000.
- The APO B value of cases is 98.79 mg/dl and controls is 84.48mg/dl, p=0.004.
- The mean APOB /APO A1 RATIO value of cases is 1.29 mg/dl and controls is 0.74,p =0.0000

# COMPARISON BETWEEN CASES AND CONTROLS ON SERUM HDL , APO A1,APO B , APO B/ APO A1 RATIO

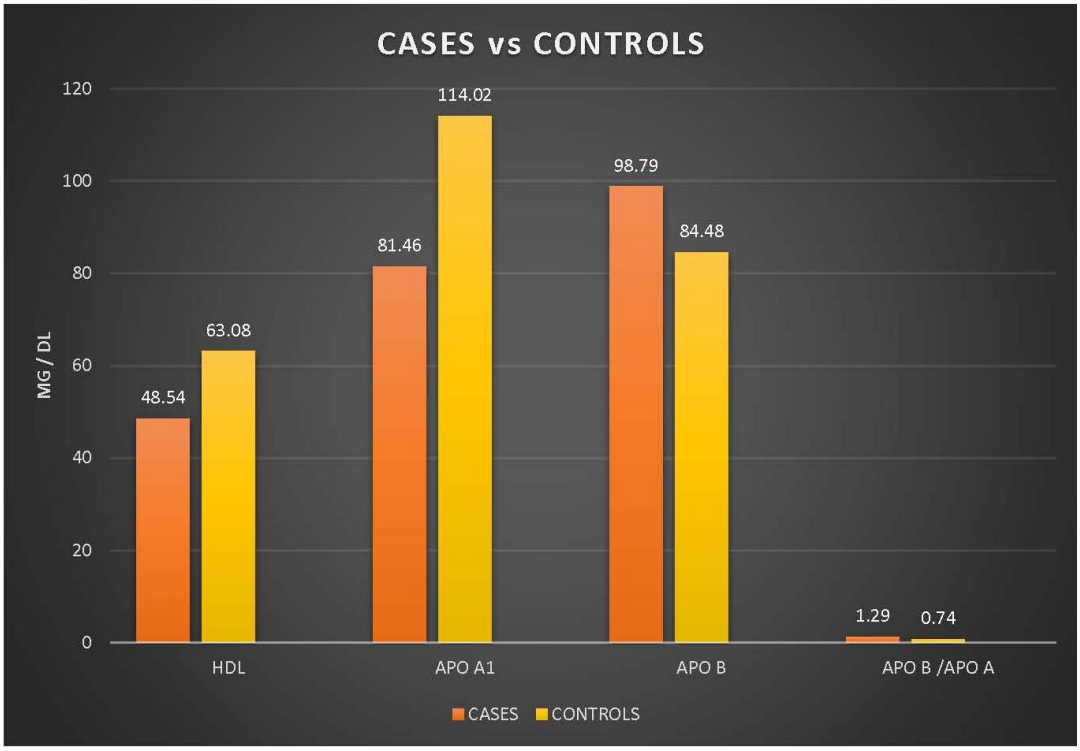


**TABLE 5**

**GENOTYPE DISTRIBUTION OF APO A1 C+83T (FIRST INTRON)IN MI CASES AND CONTROLS**

| | CC (n%) | CT(n%) | TT(n%) |
|---|---|---|---|
| CASES (n – 52) | 31 ( 59%) | 4 (8%) | 17 (33%) |
| CONTROLS (n – 52) | 42 (81%) | 6 (11%) | 4 (8%) |
| TOTAL (104) | 73 (70.19%) | 10 (9.61%) | 21 (20.19%) |

The GENOTYPE FREQUENCY were

CASES : CC-59% , CT- 8%, TT - 33%.

CONTROLS :CC- 81 % , CT- 11 %, TT - 8 %.

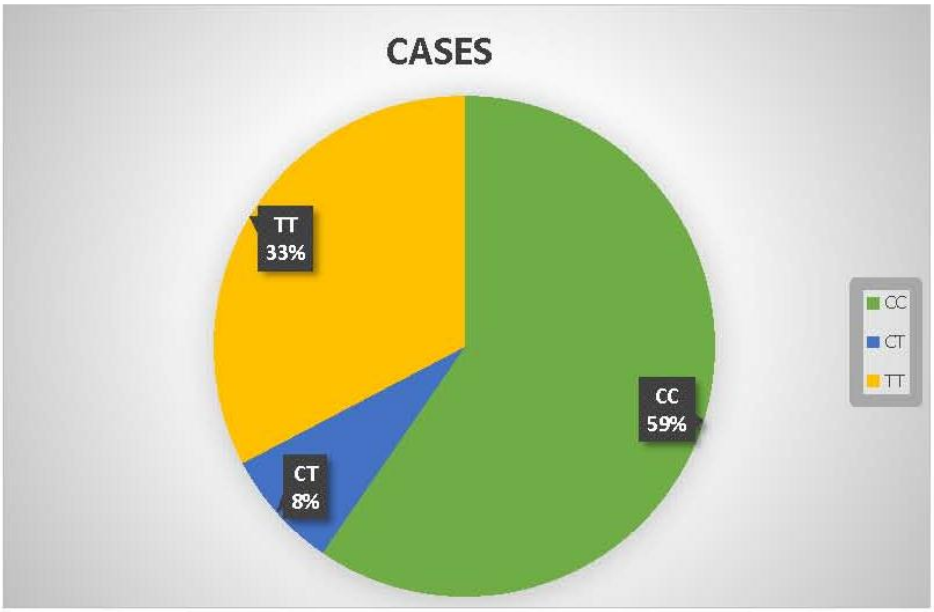
This was found to be in Hardy Weinberg equilibrium,

C Allele Frequency- Cases:0.63; Controls-0.86

T Allele Frequency- Cases:0.36;Controls-0.13

$X^2$ value- 10.105, p value is 0.006 (S)

# PIE CHART SHOWING GENOTYPEC+83T (FIRST INTRON) DISTRIBUTION IN THE STUDY POPULATION



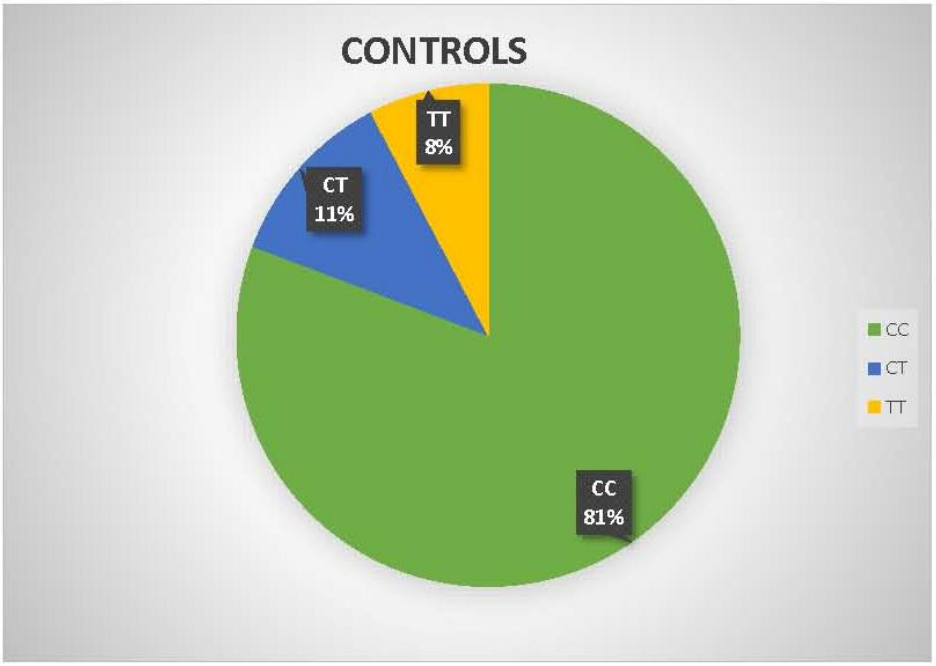



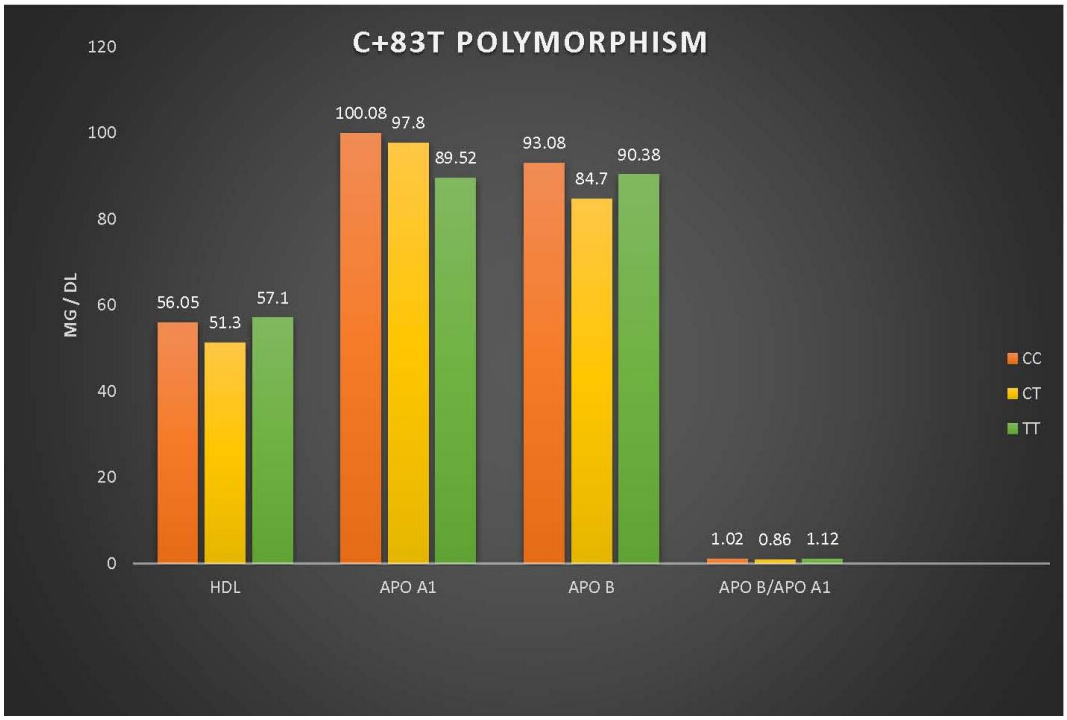
**TABLE:6**

**COMPARISON OF HDL, APO A1, APO B , APO B/APO A1 LEVELS IN DIFFERENT GENOTYPES AT +83 BASE PAIRS INDIVIDUALLY**

| In mg/dl | CC | CT | TT | p VALUE |
|---|---|---|---|---|
| HDL | 56.05 ±13.59 | 51.3±9.522 | 57.10±21.375 | 0.036 (S) |
| APO A1 | 100.08±26.707 | 97.80±15.843 | 89.52±26.726 | 0.572 (NS) |
| APO B | 93.08±23.703 | 83.70±24.363 | 90.38±31.385 | 0.080 (NS) |
| APO B / APO A1 | 1.02±0.480 | 0.86±0.267 | 1.12±0.564 | 0.167 (NS) |

- The mean HDL value across genotypes ,CC is 56.05mg/dl, CT-51.3 and TT -57.10 with p= 0.036
- The mean APO A1 value across genotypes ,CC is 100.08 mg/dl, CT-97.8 and TT –89.52 with p= 0.572
- The mean APO B value across genotypes ,CC is 93.08 mg/dl, CT- 83.7 and TT – 90.38with p= 0.080
- The mean APO B/APO A1 Ratio value across genotypes ,CC is 1.02, CT- 0.86 and TT – 1.12 with p= 0.167

# BAR DIAGRAM SHOWING GENOTYPE DISTRIBUTION AND SERUM LEVELS HDL , APO A1 AND APOB



## TABLE 7

## GENOTYPE DISTRIBUTION OF APO A1 G–75A (PROMOTER)

| | GG(n%) | GA(n%) | AA(n%) |
|---|---|---|---|
| **CASES (n – 52)** | 30 (58%) | 18 (34%) | 4 (8%) |
| **CONTROLS (n – 52)** | 23 (44%) | 26 (50%) | 3 (6%) |
| **TOTAL ( n- 104)** | 53 (50.59%) | 44 (42.43%) | 7 (6.73%) |

**REGION)INMI CASES AND CONTROLS**

The GENOTYPE FREQUENCY were

CASES: GG -58% , GA- 34%, AA - 8%.

CONTROLS: GG- 44 % , GA- 50 %, AA - 6 %.

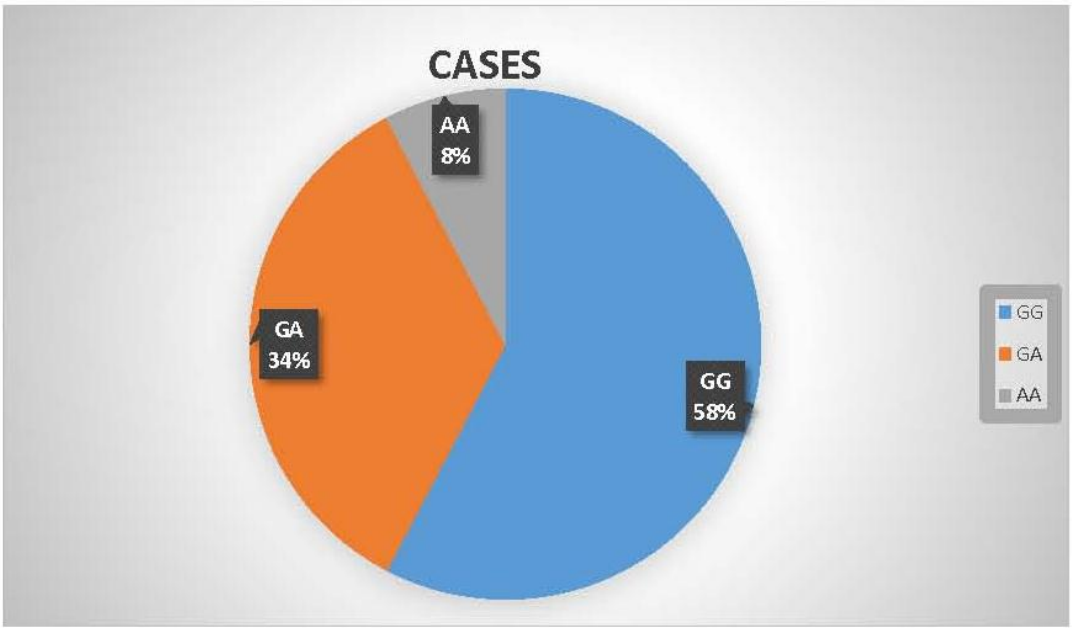
This was found to be in Hardy Weinberg equilibrium,

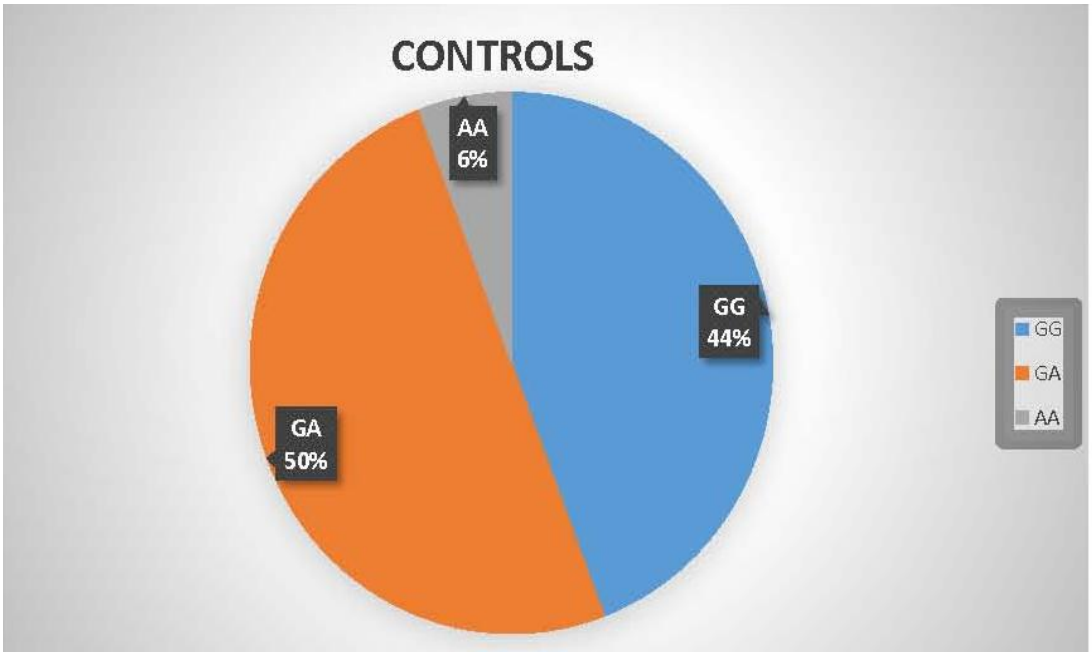
G Allele Frequency- Cases:0.75; Controls-0.69

A Allele Frequency- Cases:0.25;Controls-0.30

$X^2$ value- 2.522 , p value is 0.283(NS)

# PIE CHART SHOWING GENOTYPE DISTRIBUTION OF APO A1 G–75A (PROMOTER REGION) IN MI CASES AND CONTROLS



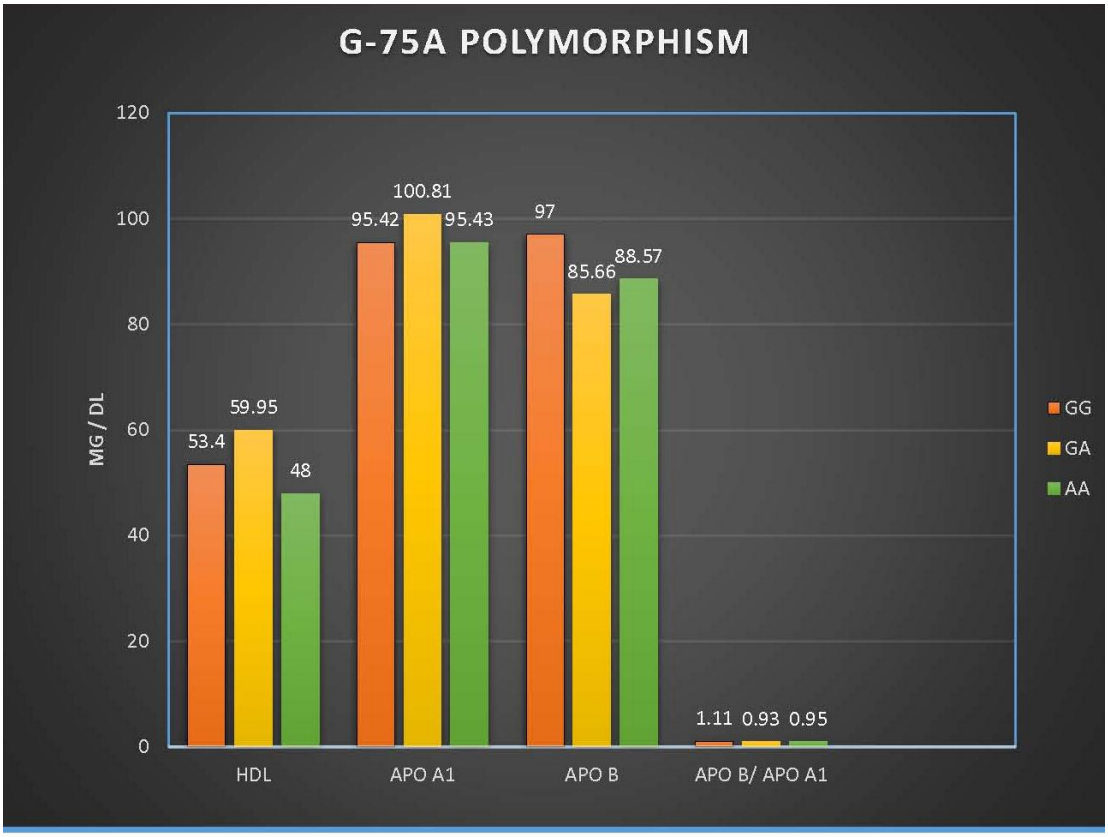
## TABLE:8

## COMPARISON OF HDL, APO A1, APO B ,APO B/APO A1 LEVELS IN DIFFERENT GENOTYPES AT - 75 BASE PAIRS INDIVIDUALLY

| | GG | GA | AA | p VALUE |
|---|---|---|---|---|
| HDL | 53.40±11.404 | 59.95±18.406 | 48.00±10.583 | 0.592 (NS) |
| APO A1 | 95.42±23.891 | 100.91±29.479 | 95.43±17.803 | 0.263(NS) |
| APO B | 97.00±22.374 | 85.66±28.498 | 88.57±19.121 | 0.535(NS) |
| APO B/ APO A1 | 1.11±0.542 | 0.93±0.419 | 0.95±0.247 | 0.390(NS) |

- The mean HDL value across genotypes ,GG is 53.40mg/dl, GA-59.95 and AA – 48.00 with p= 0.592 (NS)
- The mean APO A1 value across genotypes , GG is 95.42 mg/dl, GA -100.91 and AA– 95.43 with p= 0.263 (NS)
- The mean APO B value across genotypes ,GG is 97.00 mg/dl, GA - 85.66 and AA – 88.57 with p= 0.535 (NS)
- The mean APO B/APO A1 Ratio value across genotypes , GG - 1.11, GA - 0.93 and AA – 0.95 with p= 0.39 (NS)

**BAR DIAGRAM SHOWING COMPARISON OF HDL,APO A1,APO B AND APO B/APO A1 LEVELS IN DIFFERENT GENOTYPES AT - 75 bp**



# DISCUSSION

Many epidemiological studies have established that increased levels of Apo AI are associated with reduced occurrence and severity of CAD[83]. Several studies have analysed the possible involvement of genetic variations in the Apo A1- CIII-AIV locus in determining phenotypes and CHD. The methodology more widely used was the association of restriction fragment length polymorphisms (RFLPs) with lipids, lipoprotein, and apolipoprotein levels, and CHD.

The hypothesis in the current study is to establish the relationship between two polymorphisms **C+83T (FIRST INTRON) AND G–75A (PROMOTER REGION)**in Apo AI gene with lipid profile and occurrence of MI. At both these sites single nucleotide replacements effect in loss of MspI restriction sites so that both polymorphic sites are detected with PCR and MspI digestion in a single run. Both nucleotide changes are at regulatory hot spots. The single nucleotide change at -75 bp (promoter region) is found to be associated with altered apo AI transcription[81,82]and the base change at +83 bp(in the first intron )is found to alter either transcription or translation but the mechanism behind this has not yet been established [82].

This study aimed to look for individuals with the single nucleotide change at the above two mentioned sites are likely to have a reduced risk of developing MI.

The ApoAI-CIII-AIV gene cluster is approx 15Kb. Apo A1 gene has three introns . Apo A1 gene is disturbed by three intervening sequences (IVS) , IVS-1, IVS-2, and IVS-3[1] in the DNA region codes for the signal peptide of Apo A1. APO A1 gene has tissue specific expression in hepatocyte and intestine. Methylation status of the 5'-end of the apo-AI gene is indicative of its tissue-specific activity and 3'-end flanking sequences of the gene are invariably hypomethylated in all tissues due to an unusual characteristic of the presence of a CpG island at the 3'-end coding sequence. The DNA region between the nucleotides - 256 and - 41 upstream from the transcription start site of the human Apo A1 gene is essential and adequate for maximal expression.[76]

The apo A-I gene promoter contains a TATA-like motif close to the (25-30 bp upstream) transcriptional start site. Also Apo A1 gene is regulated by promoter/enhancer sequences containing closely spaced *cis*-acting elements on which different combinations of hepatocyte-enriched and ubiquitous factors assemble in the cis regulating elements in either a positive/negative manner in response to changes in the

hormonal or metabolic status, diet and environmental factors in the liver and intestine.

In hepatocytes, ApoAI gene transcription is maintained by three *cis*-acting elements, sites A (–214 to –192), B (–169 to –146) and C (–134 to –119), within the enhancer. Sites A and C are bound by members of the nuclear receptor superfamily. Site B binds the hepatocyte-enriched factors and C/EBP. These factors bind independently to their corresponding sites, they stimulate apoAI enhancer activity synergistically via conjoint recruitment of an uncharacterized transcriptional co-activator(s). Apo A1gene transcription is also induced by PPARα which interacts with the positive PPRE located in the A site of Apo A1 gene promoter liver specific enhancer region.

The study group included 52 patients who had documented MI, and the control group included 52 age, gender and risk factor matched groups. Fasting serum levels of TC, TGL, LDL ,HDL, Apo A1, Apo B & ratio of Apo B/ Apo A1 were estimated . TC, TGL,LDL level was significantly higher in cases compared with controls. Serum HDL, apo A1 level was significantly lower in the cases than in the control group and that the serum apo B level and apoB/apoA1ratio in the cases was

significantly higher than in the control group are consistent with the findings of various other studies.[11,19,21,83]

Polymorphisms at +83 bp and -75bp of the Apo A1 gene have been related to elevated levels of HDL cholesterol & ApoA1 in some studies[85], although not confirmed in all studies[86].

## C+83T(FIRST INTRON) POLYMORPHISM:

The comparison of genotypes across the cases and controls reveals that 'TT' homozygous genotype was higher among cases and 'CC' genotype was seen more in controls, CT more or less equal among cases and controls. Genotype difference between cases and controls were statistically significant (*p* - 0.006). As far as frequency, 'T' allele was higher among cases (0.36) as compared to controls (0.13); and frequency of 'C' allele was higher among controls (0.86) as compared to cases (0.63). Such findings were in concordance with studies conducted in Indian populations[82,,87,88] and from other subjects[86,95] where the 'T' allele was found to be higher in frequency among CAD population .This study is in discordance to some previously reported data particularly studies carried out in kashmiris,[89] Australian populations[81]. In CC genotype high mean value of Apo A1and low Apo B/Apo A1 ratio as

compared to TT genotype. But no statistically significant differences were observed between C allele and T allele carriers for any lipid variables other than HDL similar to study conducted in Brazilian children[96].

In this studied group of MI, Single base change at C+ 83T bp of TT genotype is more common in cases as compared to controls. The mechanism behind the association between the single base substitution ie C to T at +83 in the first intron of Apo A1 gene and an elevated HDL cholesterol level is yet to be finalised and requires further refinement .[82] It is suggested that the methylation pattern of the 5′ region of the APOA1 gene reveals the extent of its expression[75].In apo A1gene expressing tissues, the 5′ region of the gene is hypomethylated , ie in liver but in non expressing tissues it is heavily methylated. The MspI restriction site at +83 bp is found to contain a CpG dinucleotide. CpG dinucleotide is known to be methylated in nonexpressing cells but undermethylated in cells expressing apo A1.Possibly it could be T substitution at this site leads to further demethylation, resulting in Apo A1 gene expression. One more explanation is that the T substitution, in the 5′ end leader region for APOA1 mRNA influences the translation of

APOA1 messenger RNA(mRNA) and this could be important for the initiation of translation of mRNA.

In this study the discrepancy is due to differences in the genetic susceptibility between different ethnic groups. Also, the APOA1 gene locus lies in a cluster with CIII and AIV loci, which could be in linkage disequilibrium with the APOA1 alleles in some populations but not in others.

## G-75A( PROMOTER REGION ) POLYMORPHISM:

The comparison of genotypes across the cases and control reveals that 'GG' homozygous genotype was higher among cases and 'GA' genotype was seen more in controls, AA more or less equal among cases and controls. But the difference was statistically insignificant. As far as frequency, 'G' allele was higher among cases (0.75) as compared to controls (0.69); and frequency of 'A' allele was higher among controls (0.30) as compared to cases (0.25). Such findings were in concordance with studies conducted in Indian populations[82,,87,88] and from other subjects[86] where the 'A' allele was found to be higher in frequency among control groups. The allele frequency however was also insignificant in those studies. This study is in discordance to some

previously reported data particularly studies carried out in kashmiris ,[89] Australian populations [81]and by Reguero et al[86]with high frequency of the A allele in subjects younger than50 years with a diagnosed MI

In GA genotype mean HDL, Apo A1 were high and low Apo B/Apo A1 ratio as compared to GG genotype. But no statistically significant differences were observed between G allele and A allele carriers for any lipid variables .

The MspI -75(G/A) promoter polymorphism was first recorded by Pagani et al.[92]A potential description for the effect of the A allele on apo A1 levels suggests that the presence of the A allele at–75 bp from transcription start site of the gene increases the transcriptional efficiency of the promoter.[91]

- The allele A possibly would be in linkage disequilibrium with a different polymorphic site in the nearby gene,[92] which in turn might impact the HDL levels.

- The polymorphic site might be in a sequence with the Apo A1 gene promoter that regulates its expression in response to hormonal stimulation and by various metabolic signalling pathways[92,57,58].

- The probable mechanism is that it may be due to decreased stability of a DNA-protein complex that inhibits transcription.[82] The nucleotide region between 199 to –40 bpis needed for repression of transcription by the binding factor (MWt90 kDa) and is present in all cells that do not express the apo A1 gene other than liver and intestine in which the gene is expressed . The nucleotide at the **–75** position of the *APOA1* promoter lies in a GC-rich sequence (5′GCC[A/G]GGG-3′).The transcription of these promoters has been shown to be negatively regulated by this GC box. A GC binding factor(90-kDa factor) could mediate the repression of transcription observed in the G allele. The G to A transition decreases its binding affinity to–75 bp position and alleviates the repression of APOA1 gene transcription.

- Previous studies have reported the effect of genetic and environmental factors on Apo AI and HDL-cholesterol levels, however, the gene–environment interactions are still incompletely known[93].

# SUMMARY

Study conducted in 52 MI cases and 52 controls to look for the association between two MspI restriction sites with the lipid variables and MI, shows statistically significant differences between cases and controls were obtained, across genotype it is summarised below.

I. C+83T (FIRST INTRON) : Revealed TT genotype more in cases and CC genotype more in controls (p-0.006). In CC genotype mean value of HDL and Apo A1 were high and low Apo B/Apo A1 ratio as compared to TT genotype . T allele frequency is more in cases and C allele more in controls. Difference in the mean for lipid variables were obtained between C allele subjects and T allele carriers but it was not statistically significant except HDL.

II. G-75A (PROMOTER) : Revealed GG genotype was more in cases and GA genotype more in controls, AA genotype distribution is more or less equal between cases and control. No statistically significant difference across genotypes were obtained. In GA genotype mean value of HDL, Apo A1 were high and low Apo B/Apo A1 ratio as compared to GG genotype. G allele frequency is more in cases and A allele more in controls. No statistically

significant differences were observed between G allele and A allele carriers for any lipid variables.

In this study Statistically significant difference could not be obtained for both the polymorphic site , other than HDL in C+83T because

- It is critically important to consider the level of confidence to place the accuracy of results.
- Genetic susceptibility between different ethnic groups.
- The MspI polymorphisms at two sites could be in linkage disequilibrium with each other , or with near by gene or with CIII and AIV cluster. [85] .
- The genotype effect on circulating Apo A1 and HDL cholesterol levels is modulated by gender, hormonal and various metabolic signalling pathways as in DM, diet and environmental factors such as smoking [57,58].
- Small population chosen.
- Frequency of the presence of rare alleles.

# CONCLUSION

- In this study T allele is significantly increased in cases compared to controls which implies that the presence of T allele present in C+83T (first intron) of the Apo A1 gene, might increase the development of MI. When compared across the lipid variables , no significant difference were obtained other than HDL, since various factors influences the Apo A1 gene expression.

- In this study, no statistically significant differences were obtained across genotype as well as lipid variables in G-75A (promoter) region of Apo A1 gene for the development of MI.

# LIMITATIONS

Limitations of this study are

- Small sample size .
- Probable occurrence of linkage disequilibrium of APO AI gene with the near by polymorphic site and the gene complex APO CIII and APO AIV.
- Gender, hormonal and various metabolic signalling pathways as in DM , diet and environmental factors such as smoking could have modulated the genotype effect on circulating Apo A1 and HDL for the inheritance of such a complex trait CAD which progress to dreadful complication MI.
- Low frequency of the presence of rare alleles.

# SCOPE FOR FURTHER STUDY

- Genotypic differences across different ethnic groups for this two polymorphic site has to be established.

- More studies with larger samples are needed to confirm genotypic risk associated with the both the polymorphic sites in the development of MI

- Further research may focus upon the gene – gene interactions and gene environmental interactions and its relationship with the genotypic variation of Apo A1 gene in patients with MI so as to provide efficient preventive measures to genetically susceptible population in future.

- Probable occurrence of linkage disequilibrium of APO AI gene with the near by polymorphic site and the gene complex APO CIII and APO AIV has to be established.

- Whole genome sequence analysis is needed to reveal extensive level of variation and heterogeneity between individuals and populations and genome-wide association studies (GWAS) has to be done , as these analyses eliminates biases in the selection of the candidate genes.

# PROFORMA

**Date**:

**Name:** **OP/IP No:**

**Age:** **Sex :**

**Address:** **Occupation:**

**Presenting Complaints:**

**Past H/O**:

DM/ HT / AGE AT ONSET OF CAD/ Liver disease /Renal disease

**Treatment H/O:**

STATIN THERAPY / BETA BLOCKERS / ACE INHIBITORS/ CALCIUM CHANNEL BLOCKERS / HYPOGLYCEMIC AGENTS

**Previous hospitalization:**

**Personal H/O:**

SMOKING / ALCOHOL INTAKE

**O/E:**

Built -

Height-

Pedal edema /Anemia/Clubbing /Lymphadenopathy

**VITALS:**

BP:

Pulse Rate:

**SYSTEMIC EXAMINATION :**

CVS: Abdomen :

RS : CNS:

**DIAGNOSIS:**

**INVESTIGATIONS:**

1.Blood sugar2. Urea3. Creatinine

4. Fasting lipid profile :

- Total Cholesterol
- Triglyceride
- High Density Cholesterol (HDL-c)
- Low Density Cholesterol (LDL-c)
- Apolipoprotein A1 (Apo A1)
- Apolipoprotein B100 (Apo B)

5. Genetic polymorphic studies

- DNA EXTRACTION
- GENOMIC DNA AMPLIFICATION BY PCR
- 2% AGAROSE GEL RUN
- RESTRICTION DIGESTION OF THE PCR PRODUCT
- Genotype :GG/GA/AA

  CC/CT/TT

# நோயாளி ஒப்புதல் படிவம்

ஆராய்ச்சியின் விவரம்: இருதய நோயாளிகள் மற்றும் இதர சாதாரண மக்களுக்கும் உள்ள apo A1 மரபணு பல்லுருத்தோற்றத்தினால் உண்டான தொடர்பு.

ஆராய்ச்சி மையம்: அரசு கீழ்ப்பாக்கம் மருத்துவக் கல்லூரி மருத்துவமனை

நோயாளியின் பெயர்: நோயாளியின் வயது:

பதிவு எண்: முகவரி:

1. மேற்குறிப்பிட்டுள்ள ஆராய்ச்சியின் நோக்கத்தையும், பயனையும் முழுவதுமாக புரிந்துக்கொண்டேன். மேலும் எனது அனைத்து சந்தேகங்களையும் கேட்டு அதற்கான விளக்கங்களையும் தெளிவுபடுத்திக் கொண்டேன்.
2. மேலும் இந்த ஆராய்ச்சிக்கு எனது சொந்த விருப்பத்தின் பேரில் பங்கேற்கிறேன் என்றும், மேலும் எந்த நேரத்திலும் எவ்வித முன்னறிவிப்புமின்றி இந்த ஆராய்ச்சியிலிருந்து விலக முழுமையான உரிமை உள்ளதையும், இதற்கு எவ்வித சட்ட பிணைப்பும் இல்லை என்பதையும் அறிவேன்.
3. ஆராய்ச்சியாளரோ, ஆராய்ச்சி உதவியாளரோ, ஆராய்ச்சி உபயத்தாரோ, ஆராய்ச்சி பேராசிரியரோ, ஒழுங்கு நெறி செயற்குழு உறுப்பினர்களோ எப்போது வேண்டுமானாலும் எனது அனுமதியின்றி எனது உள் நோயாளி பதிவுகளை / எஞ்சியுள்ள மரபணுவையும் இந்த ஆராய்ச்சிக்காகவோ, அல்லது எதிர்கால பிற ஆராய்ச்சிக்களுக்காகவோ பயன்படுத்திக் கொள்ளலாம் என்றும் மேலும் இந்த நிபந்தனை நான் இவ்வாராய்ச்சியிலிருந்து விலகினாலும் தகும் என்றும் ஒப்புக்கொள்கிறேன். ஆயினும் எனது அடையாளம் சம்பந்தப்பட்ட எந்த பதிவுகளும் (சட்ட பூர்வமான தேவைகள் தவிர) வெளியிடப்படமாட்டாது என்ற உறுதிமொழியின் பெயரில் இந்த ஆராய்ச்சியிலிருந்து கிடைக்கப்பெறும் முடிவுகளை வெளியிட மறுப்பு தெரிவிக்கமாட்டேன் என்று உறுதியளிக்கின்றேன்.
4. இந்த ஆராய்ச்சிக்கு நான் முழுமனதுடன் சம்மதிக்கின்றேன் என்றும் மேலும் ஆராய்ச்சிக் குழுவினர் எனக்கு அளிக்கும் அறிவுரைகளை தவறாது பின்பற்றுவேன் என்றும் உறுதியளிக்கின்றேன்.
5. இந்த ஆராய்ச்சிக்குத் தேவைப்படும் அனைத்து மருத்துவர் பரிசோதனைகளுக்கும் ஒத்துழைப்பு தருவேன் என்று உறுதியளிக்கிறேன்.
6. இந்த ஆராய்ச்சியில் இருதய நோய்க்கான பரிசோதனைகளுக்கும் மேலும் மரபணு சோதனையும் மேற்கொள்ளப்படுகிறது என்பதை ஆராய்ச்சியாளர்கள் மூலம் அறிந்து கொண்டேன். மரபணு சோதனைக்கும் எனது முழு ஒப்புதலை தருகிறேன்.
7. இந்த ஆராய்ச்சிக்கு யாருடைய வற்புறுத்தலுமின்றி எனது சொந்த விருப்பத்தின் பேரிலும் சுய அறிவுடனும் முழுமனதுடனும் சம்மதிக்கின்றேன் என்று இதன் மூலம் ஒப்புக்கொள்கிறேன்.

நோயாளியின் கையொப்பம் / பெருவிரல் கைரேகை

இடம்: தேதி:

ஆராய்ச்சியாளர் கையொப்பம்:

இடம்: தேதி:

# MASTER CHART – CASES

| Sl.No | PATIENT NAME | AGE | GENDER | SMOKER | ALCO HOL | H/O CAD | HT | DM | Glu | Urea | Creat | Chol | TGL | HDL | LDL | APO A1 | APO B | APO B/APO A | G-75A | C+83T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T1 | SIVARAJ | 43 | M | Y | Y | N | Y | Y | 108 | 23 | 0.8 | 174 | 185 | 35 | 117 | 68 | 109 | 1.60 | GA | CC |
| T2 | BALAMURUGAN | 45 | M | Y | Y | N | N | Y | 109 | 14 | 1.07 | 206 | 129 | 62 | 96 | 88 | 99 | 1.13 | GA | CT |
| T3 | KARUPPASAMY | 50 | M | Y | Y | N | N | Y | 139 | 44 | 1.9 | 192 | 144 | 55 | 95 | 92 | 104 | 1.13 | GA | CC |
| T4 | HASIBUL | 56 | M | N | N | N | N | Y | 92 | 42 | 0.95 | 133 | 169 | 41 | 66 | 113 | 88 | 0.78 | AA | CT |
| T5 | MILLER | 42 | M | Y | Y | N | N | y | 317 | 28 | 6 | 216 | 165 | 24 | 101 | 24 | 59 | 2.46 | GA | CC |
| T6 | VELU | 58 | M | Y | Y | N | N | N | 121 | 26 | 1.17 | 220 | 155 | 75 | 122 | 95 | 111 | 1.17 | GA | CC |
| T7 | BAHADUR | 56 | M | N | N | Y | N | N | 100 | 15 | 0.81 | 121 | 93 | 44 | 46 | 82 | 71 | 0.87 | GG | CC |
| T8 | VADIVELU | 67 | M | Y | N | N | N | N | 98 | 17 | 0.94 | 179 | 130 | 62 | 89 | 115 | 94 | 0.82 | GG | CC |
| T9 | VANI | 42 | F | N | N | N | Y | N | 104 | 18 | 0.64 | 198 | 149 | 64 | 109 | 117 | 113 | 0.97 | GA | CC |
| T10 | KRISHNAN | 51 | M | N | Y | N | N | N | 79 | 40 | 1.4 | 178 | 118 | 60 | 85 | 73 | 89 | 1.22 | GG | CC |
| T11 | VIJAYAKUMAR | 46 | M | N | N | Y | N | N | 114 | 19 | 1.1 | 154 | 142 | 43 | 58 | 73 | 85 | 1.16 | GA | CC |
| T12 | JOHNY BASHA | 52 | M | N | N | N | N | Y | 322 | 41 | 0.92 | 309 | 276 | 30 | 84 | 68 | 79 | 1.16 | AA | CC |
| T13 | KRISHNAIAH | 58 | M | Y | N | N | N | Y | 199 | 39 | 1.31 | 419 | 264 | 45 | 97 | 53 | 65 | 1.23 | GA | CC |
| T14 | PARANTHAMAN | 62 | M | N | N | N | N | Y | 246 | 37 | 1.48 | 197 | 156 | 51 | 135 | 73 | 109 | 1.49 | GG | CC |
| T15 | PALANIVEL | 58 | M | N | N | Y | N | N | 61 | 35 | 1.18 | 161 | 134 | 71 | 90 | 119 | 101 | 0.85 | GG | CC |
| T16 | MNUISAD ALI | 42 | M | N | N | N | Y | N | 102 | 22 | 1.01 | 145 | 210 | 41 | 73 | 29 | 108 | 3.72 | GG | CC |
| T17 | KOLLAPURI | 42 | M | Y | Y | N | N | Y | 167 | 16 | 0.95 | 188 | 132 | 39 | 121 | 70 | 104 | 1.49 | GG | CC |
| T18 | KUMAR | 55 | M | N | N | N | N | Y | 157 | 191 | 5.79 | 202 | 232 | 43 | 122 | 83 | 143 | 1.72 | GG | CC |
| T19 | ASHOKKUMAR | 56 | M | N | N | N | Y | Y | 102 | 37 | 1.82 | 184 | 267 | 44 | 70 | 76 | 93 | 1.22 | GG | CT |
| T20 | ANBUMANI | 57 | M | N | N | N | N | N | 63 | 30 | 1.07 | 249 | 268 | 46 | 167 | 101 | 136 | 1.35 | GG | CC |
| T21 | ANAND | 53 | M | Y | N | N | N | N | 84 | 26 | 0.98 | 179 | 232 | 40 | 83 | 74 | 113 | 1.53 | GA | CC |
| T22 | SHAHUL HAMEED | 62 | M | N | N | Y | N | N | 72 | 28 | 0.86 | 210 | 133 | 53 | 130 | 105 | 123 | 1.17 | AA | CT |
| T23 | SARASWATHY | 59 | F | N | N | N | N | Y | 138 | 31 | 0.94 | 315 | 212 | 78 | 202 | 117 | 154 | 1.32 | GG | TT |
| T24 | MURUGESAN | 60 | M | N | N | N | Y | Y | 137 | 38 | 1.12 | 181 | 153 | 43 | 93 | 97 | 139 | 1.43 | GA | CC |
| T26 | LOURDASAMY | 51 | M | Y | N | N | Y | Y | 225 | 30 | 0.99 | 270 | 141 | 51 | 105 | 90 | 135 | 1.50 | GA | CC |
| T25 | VENUOPAL | 44 | M | N | Y | N | N | Y | 264 | 13 | 0.9 | 159 | 196 | 59 | 109 | 86 | 88 | 1.02 | GA | CC |
| T27 | RAVINDRANATH | 62 | M | N | N | N | N | Y | 192 | 25 | 2.7 | 261 | 129 | 47 | 108 | 81 | 103 | 1.27 | AA | TT |

# MASTER CHART – CASES

| Sl.No | PATIENT NAME | AGE | GENDER | SMOKER | ALCOHOL | H/O CAD | HT | DM | Glu | Urea | Creat | Chol | TGL | HDL | LDL | APO A1 | APO B | APO B/APO A | G-75A | C+83T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T28 | PERIASAMY | 70 | M | Y | N | N | N | N | 117 | 70 | 2.46 | 78 | 214 | 24 | 136 | 42 | 59 | 1.40 | GA | CC |
| T29 | RAVI | 48 | M | N | Y | N | N | N | 55 | 18 | 0.99 | 198 | 187 | 44 | 169 | 70 | 108 | 1.54 | GG | CC |
| T30 | GEORGE | 61 | M | N | N | N | Y | N | 91 | 14 | 0.99 | 160 | 108 | 52 | 115 | 83 | 82 | 0.99 | GG | TT |
| T31 | GUNASEKARAN | 49 | M | Y | N | Y | N | N | 88 | 21 | 0.78 | 77 | 106 | 29 | 42 | 48 | 49 | 1.02 | GG | CC |
| T32 | BASHEER AHAMED | 57 | M | N | N | Y | N | N | 128 | 16 | 1.18 | 187 | 103 | 46 | 131 | 72 | 100 | 1.39 | GG | CC |
| T33 | THIRUVENGADAM | 59 | M | N | N | N | N | Y | 137 | 21 | 1.03 | 225 | 169 | 49 | 155 | 88 | 102 | 1.16 | GG | CC |
| T34 | MANGALAKSHMI | 70 | F | N | N | N | Y | Y | 397 | 26 | 1 | 166 | 103 | 54 | 108 | 86 | 87 | 1.01 | GG | CC |
| T35 | ARUMUGAM | 47 | M | N | N | N | Y | Y | 89 | 14 | 2.51 | 213 | 149 | 43 | 145 | 104 | 114 | 1.10 | GA | CC |
| T36 | RAJA | 43 | M | N | N | N | Y | N | 68 | 19 | 0.49 | 228 | 100 | 59 | 128 | 102 | 90 | 0.88 | GG | CC |
| T37 | LAKSHMI | 63 | F | N | N | N | Y | N | 95 | 32 | 1.03 | 238 | 126 | 47 | 167 | 85 | 120 | 1.41 | GG | CC |
| T38 | PRABHU | 54 | M | N | Y | N | N | Y | 143 | 16 | 0.9 | 202 | 153 | 59 | 134 | 102 | 135 | 1.32 | GG | TT |
| T39 | MOORTHY | 50 | M | N | Y | N | N | N | 78 | 19 | 0.78 | 150 | 181 | 53 | 71 | 100 | 62 | 0.62 | GG | TT |
| T40 | PONNIAH | 62 | M | N | N | N | N | Y | 359 | 77 | 2.65 | 150 | 139 | 36 | 67 | 69 | 77 | 1.12 | GG | TT |
| T41 | KARTHIK | 41 | M | Y | Y | N | N | N | 115 | 17 | 0.9 | 165 | 159 | 58 | 108 | 97 | 86 | 0.89 | GA | CC |
| T42 | BHARATHI | 62 | F | N | N | Y | N | N | 127 | 22 | 0.75 | 182 | 91 | 53 | 186 | 98 | 75 | 0.77 | GG | TT |
| T43 | VAIDHEESWARAN | 53 | M | N | N | N | N | N | 84 | 15 | 0.41 | 233 | 113 | 22 | 103 | 37 | 42 | 1.14 | GA | TT |
| T44 | BALAKRISHNAN | 59 | M | Y | Y | N | N | N | 77 | 19 | 1.05 | 194 | 130 | 58 | 173 | 84 | 115 | 1.37 | GA | TT |
| T45 | KATHIRVEL | 44 | M | N | N | N | N | Y | 152 | 26 | 0.83 | 229 | 368 | 35 | 247 | 44 | 133 | 3.02 | GG | TT |
| T46 | IQBAL | 52 | M | N | N | N | Y | N | 124 | 15 | 0.8 | 204 | 177 | 57 | 153 | 90 | 105 | 1.17 | GG | TT |
| T47 | SUBRAMANI | 42 | M | Y | Y | N | N | N | 100 | 19 | 1.01 | 101 | 312 | 55 | 181 | 88 | 93 | 1.06 | GG | TT |
| T48 | GOVARDHAN | 62 | M | Y | Y | N | Y | Y | 90 | 24 | 0.8 | 249 | 135 | 23 | 37 | 50 | 59 | 1.18 | GG | TT |
| T49 | KOTHAWARI | 42 | F | N | Y | N | N | N | 114 | 40 | 0.96 | 207 | 165 | 45 | 177 | 73 | 132 | 1.81 | GG | TT |
| T50 | MOHAMED | 71 | M | N | N | Y | N | N | 89 | 14 | 0.89 | 236 | 81 | 61 | 130 | 85 | 92 | 1.08 | GA | TT |
| T51 | DHANAM | 52 | F | N | N | N | N | Y | 155 | 38 | 0.67 | 224 | 67 | 63 | 153 | 85 | 103 | 1.21 | GG | TT |
| T52 | VENKATESH RAO | 54 | M | N | Y | N | N | N | 100 | 32 | 1.19 | 170 | 185 | 53 | 173 | 92 | 102 | 1.11 | GG | TT |

# MASTER CHART – CONTROLS

| SLNO | PATIENT NAME | AGE | GENDER | SMOKER | ALCOHOL | H/O CAD | HT | DM | Glu | Urea | Creat | CHO | TGL | HDL | LDL | Apo A | Apo B | APOB/APOA | G-75A | C+83T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C1 | ARULMOZHI | 57 | M | N | N | N | N | y | 243 | 25 | 0.71 | 177 | 113 | 56 | 105 | 110 | 82 | 0.75 | GG | CC |
| C2 | KASTHURI RANGAN | 58 | M | Y | Y | N | Y | Y | 87 | 22 | 1.22 | 152 | 106 | 47 | 98 | 128 | 66 | 0.52 | GG | CC |
| C3 | GEETHA | 54 | F | N | N | N | Y | N | 69 | 23 | 0.88 | 129 | 120 | 60 | 102 | 155 | 72 | 0.46 | GA | CC |
| C4 | GANAPATHY | 65 | M | N | N | N | N | N | 90 | 17 | 1.05 | 175 | 86 | 71 | 66 | 129 | 90 | 0.70 | GG | CC |
| C5 | CHANDRA | 56 | F | N | N | N | N | N | 94 | 19 | 0.75 | 180 | 103 | 67 | 111 | 126 | 108 | 0.86 | GA | CC |
| C6 | VASUDEVAN | 42 | M | N | N | N | Y | N | 75 | 41 | 0.97 | 136 | 87 | 61 | 85 | 92 | 64 | 0.70 | AA | CC |
| C7 | ARUL | 46 | M | N | Y | N | N | Y | 315 | 30 | 0.91 | 133 | 123 | 61 | 104 | 108 | 66 | 0.61 | GG | CC |
| C8 | HARIKRISHNAN | 42 | M | N | N | N | N | N | 76 | 25 | 1.03 | 192 | 94 | 63 | 86 | 98 | 92 | 0.94 | GG | CC |
| C9 | SUBRAMANIAN | 42 | M | N | N | N | Y | N | 75 | 32 | 1.04 | 174 | 104 | 65 | 105 | 118 | 85 | 0.72 | GA | CC |
| C10 | MUTHULAKSHMI | 68 | F | N | N | N | Y | N | 116 | 41 | 0.76 | 175 | 104 | 75 | 108 | 126 | 111 | 0.88 | GA | CC |
| C11 | RANI | 48 | F | N | N | N | N | Y | 190 | 25 | 0.99 | 129 | 89 | 73 | 78 | 141 | 99 | 0.70 | GG | CC |
| C12 | RAMESH | 45 | M | N | N | N | N | Y | 274 | 30 | 1.03 | 221 | 194 | 67 | 104 | 113 | 115 | 1.02 | GG | CC |
| C13 | LEELA | 65 | F | N | N | N | N | Y | 172 | 24 | 0.73 | 170 | 102 | 82 | 104 | 137 | 69 | 0.50 | GG | CC |
| C14 | LEELAVATHY | 68 | F | N | N | N | Y | Y | 65 | 26 | 0.96 | 152 | 116 | 58 | 94 | 119 | 98 | 0.82 | GG | CC |
| C15 | NARAYANAN | 49 | M | N | N | N | N | N | 76 | 26 | 0.93 | 209 | 118 | 57 | 111 | 116 | 113 | 0.97 | GG | CC |
| C16 | RAJESHWARI | 59 | F | N | N | N | N | N | 105 | 21 | 1.08 | 221 | 118 | 63 | 112 | 126 | 118 | 0.94 | GA | CC |
| C17 | RAJESH | 42 | M | N | N | N | N | y | 127 | 23 | 0.76 | 173 | 154 | 61 | 75 | 134 | 120 | 0.90 | GG | CC |
| C18 | LAKSHMI | 33 | F | N | N | N | N | N | 95 | 17 | 0.73 | 165 | 113 | 53 | 113 | 112 | 100 | 0.89 | GG | CC |
| C19 | MARIAPPAN | 56 | M | N | N | N | N | N | 92 | 21 | 0.97 | 125 | 138 | 46 | 56 | 91 | 83 | 0.91 | AA | CC |
| C20 | NAGABOOSANAM | 40 | M | N | N | N | N | N | 72 | 18 | 0.97 | 158 | 122 | 54 | 66 | 104 | 84 | 0.81 | GG | CC |
| C21 | KRISHNAMOORTHY | 45 | M | Y | N | N | N | N | 78 | 23 | 0.83 | 213 | 135 | 60 | 138 | 119 | 132 | 1.11 | GG | CC |
| C22 | MITHRAN | 40 | M | N | N | N | N | Y | 50 | 29 | 0.97 | 97 | 124 | 42 | 53 | 87 | 92 | 1.06 | GG | CC |
| C23 | SELVAMANI | 42 | M | Y | Y | N | N | N | 101 | 41 | 0.91 | 165 | 150 | 60 | 82 | 122 | 94 | 0.77 | GG | CC |
| C24 | GNANASAMBANDAM | 59 | M | N | N | N | N | N | 76 | 24 | 1.04 | 131 | 84 | 76 | 58 | 125 | 72 | 0.58 | GA | CC |
| C25 | RAVIBALAN | 51 | M | N | N | N | N | N | 91 | 28 | 1.14 | 143 | 109 | 51 | 43 | 106 | 77 | 0.73 | GG | CT |
| C26 | RAMANI | 63 | M | Y | Y | N | Y | y | 192 | 18 | 0.94 | 197 | 195 | 58 | 97 | 112 | 100 | 0.89 | GG | CC |
| C27 | MURUGANANDHAN | 56 | M | N | Y | N | N | N | 101 | 33 | 1.03 | 194 | 85 | 57 | 108 | 104 | 96 | 0.92 | GG | CC |

# MASTER CHART – CONTROLS

| SLNO | PATIENT NAME | AGE | GENDER | SMOKER | ALCOHOL | H/O CAD | HT | DM | Glu | Urea | Creat | CHO | TGL | HDL | LDL | Apo A | Apo B | APOB/APOA | G-75A | C+83T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C28 | R.R.PAI | 72 | M | Y | N | N | N | Y | 30 | 25 | 0.78 | 93 | 73 | 71 | 27 | 115 | 56 | 0.49 | GA | CC |
| C29 | RAMANUJAM | 56 | M | N | N | N | Y | Y | 136 | 20 | 0.73 | 243 | 83 | 57 | 175 | 95 | 103 | 1.08 | GG | CT |
| C30 | BALU | 49 | M | N | Y | N | N | Y | 135 | 22 | 0.88 | 300 | 128 | 71 | 104 | 91 | 113 | 1.24 | GA | CC |
| C31 | VAIDHYANATHAN | 67 | M | N | N | N | Y | N | 99 | 21 | 0.96 | 145 | 141 | 72 | 76 | 157 | 94 | 0.60 | GA | CC |
| C32 | KALAISELVAM | 50 | M | N | Y | N | N | Y | 122 | 35 | 1.07 | 239 | 190 | 72 | 197 | 124 | 133 | 1.07 | GA | CC |
| C33 | NAJEEMOHAIDEEN | 45 | M | N | N | N | N | Y | 272 | 17 | 0.74 | 156 | 229 | 57 | 100 | 120 | 110 | 0.92 | GA | CC |
| C34 | JAYACHANDRAN | 40 | M | N | N | N | N | N | 81 | 27 | 0.88 | 173 | 187 | 62 | 135 | 106 | 97 | 0.92 | GA | CC |
| C35 | RAMAMURTHY | 74 | M | N | N | N | N | N | 114 | 15 | 0.43 | 172 | 81 | 71 | 116 | 117 | 67 | 0.57 | GA | CC |
| C36 | RAMALINGAM | 46 | M | N | N | N | N | y | 235 | 23 | 0.91 | 195 | 202 | 41 | 146 | 105 | 85 | 0.81 | GG | CC |
| C37 | ARULALAN | 69 | M | N | N | N | N | N | 62 | 22 | 0.82 | 128 | 158 | 58 | 95 | 118 | 80 | 0.68 | AA | CT |
| C38 | ANBU | 50 | M | N | N | N | N | Y | 339 | 17 | 0.95 | 151 | 150 | 55 | 97 | 98 | 75 | 0.77 | GG | CT |
| C39 | DAVID | 55 | M | N | Y | N | Y | Y | 263 | 14 | 1.27 | 195 | 240 | 61 | 150 | 106 | 89 | 0.84 | GA | CC |
| C40 | BASKARAN | 64 | M | N | N | N | N | y | 202 | 19 | 1.02 | 158 | 104 | 60 | 104 | 110 | 67 | 0.61 | GA | CT |
| C41 | SABARATHINAM | 58 | M | N | N | N | N | N | 109 | 14 | 1.13 | 195 | 233 | 63 | 141 | 107 | 77 | 0.72 | GA | CC |
| C42 | MUTHIAH | 42 | M | N | Y | N | N | N | 104 | 15 | 0.96 | 158 | 168 | 55 | 106 | 97 | 77 | 0.79 | GG | CC |
| C43 | SUBRAMANIAN | 66 | M | N | N | N | N | N | 88 | 26 | 1.01 | 245 | 151 | 87 | 204 | 130 | 89 | 0.68 | GA | CC |
| C44 | SIVA | 57 | M | N | N | N | Y | Y | 145 | 18 | 1.15 | 237 | 214 | 72 | 175 | 125 | 101 | 0.81 | GA | CC |
| C45 | GANESH | 49 | M | N | N | N | N | N | 95 | 33 | 0.92 | 123 | 135 | 32 | 17 | 70 | 32 | 0.46 | GA | CT |
| C46 | GANESAN | 60 | M | Y | Y | N | N | N | 82 | 13 | 1.01 | 119 | 100 | 76 | 87 | 115 | 47 | 0.41 | GA | CC |
| C47 | KABILAN | 48 | M | Y | Y | N | N | N | 81 | 15 | 0.99 | 161 | 101 | 64 | 96 | 104 | 66 | 0.63 | GA | TT |
| C48 | PAKKIRISAMY | 68 | M | Y | Y | N | N | N | 62 | 16 | 1.1 | 188 | 77 | 89 | 121 | 131 | 63 | 0.48 | GA | TT |
| C48 | BALU | 65 | M | Y | Y | N | N | N | 101 | 13 | 1.1 | 82 | 62 | 36 | 47 | 64 | 38 | 0.59 | GA | CC |
| C50 | ELUMALAI | 48 | M | N | Y | N | N | N | 98 | 14 | 0.74 | 176 | 101 | 81 | 122 | 126 | 53 | 0.42 | GA | TT |
| C51 | JAGAL | 43 | M | Y | N | N | N | y | 128 | 13 | 0.69 | 177 | 82 | 115 | 142 | 141 | 52 | 0.37 | GA | TT |
| C52 | VIJAYAN | 55 | M | N | N | N | N | N | 117 | 16 | 1.01 | 110 | 127 | 58 | 33 | 99 | 31 | 0.31 | GA | CC |

# BIBLIOGRAPHY


1. Karathanasis SK, McPheroson J, Zannis VI, Breslow JL. Linkage of human Apolipoprotein AI, CIII and AIV genes. Nature 1983;304:371-3.

2. Groenendijk M, Cantor RM, de Bruin TW, Dallinga-Thie GM. The ApoAI-CIII-AIV gene cluster. Atherosclerosis 2001;157: 1-11.

3. Ordovas JM, Civeira F, Genest J Jr et al. Restriction fragment length polymorphisms of the apolipoprotein A-I, C-III, A-IV gene locus. Relationships with lipids, apolipoproteins, and premature coronary artery disease. Atherosclerosis 1991;87:75-86.

4. Tybjaerg-Hansen A, Nordestgaard BG, Gerdes LU, Faergeman O, Humphries SE. Genetic markers in the apo AI-CIII-AIV gene cluster for combined hyperlipidemia, hypertriglyceridemia, and predisposition to atherosclerosis. Atherosclerosis 1993;100: 157-69.

5. Paul-Hayase H, Rosseneu M, Robinson D, Van Bervliet JP, Deslypere JP, Humphries SE. Polymorphisms in the apolipoprotein (apo) AI-CIII-AIV gene cluster: detection of genetic variation determining plasma apo AI, apo CIII and apo AIV concentrations. Hum Genet 1992;88:439-46.

6. Kee F, Amouyel P, Fumeron F. Lack of association between genetic variations of apo A-I-C-III-A-IV gene cluster and myocardial infarction in a sample of European male: ECTIM study. Atherosclerosis 1999;145: 187-95.

7. Vavatsi NA, Kouidou SA, Geleris PN. Increased frequency of the rare PstI allele (P2) in a population of CAD patients in northern Greece. ClinGenet 1995;47:22-6.10. Mooradian AD, Haas MJ, Wong NC. Transcriptional control

8. Gordon DJ, Probstfield JL, Garrison RJ, et al. High density lipoprotein cholesterol and cardiovascular disease: four prospective American studies. Circulation. 1999;79:8-15.

9. Wilson PW, Abbott RD, Castelli WP. High densitylipoprotein cholesterol and mortality: the Framingham Heart Study. Arteriosclerosis. 1998;8:737-741.

10. Ballantyne CM, Hoogeven RC. Role of lipid and lipoprotein profiles in risk assessment and therapy. Am Heart J. 2003;146:227-233

11. Packard CJ. Apolipoproteins: the new prognostic indicator? Eur Heart J Suppl. 2003;5:D9-D16.

12. Sniderman AD, Furberg CD, Keech A, et al. Apolipoproteins versus lipids as indices of coronary risk and as targets for statin treatment. Lancet. 2003;361:777-780.

13. Wallidus G, Junger I. Apolipoprotein B and apolipoprotein A-1: risk indicators of coronary heart disease and targets for lipid modifying therapy. J Intern Med. 2004;255:188-205.

14. Curtiss LK, Valenta DT, Hime NJ, et al. What is so special about apolipoprotein A1 in reverse cholesterol transport? Arterioscler Thromb Vasc Biol. 2006;26:12-19.

15. Assmann G, von Eckardstein A, Funke H. High density lipoprotein, reverse cholesterol transport and CAD: insights from mutations. Circulation. 1993;87(4 suppl):III28-III34.

16. The Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial infarction redefined—A consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. Eur Heart J 2000;21:1502–1513; J Am Coll Cardiol 2000;36:959 –969.

17. Thygesen K, Alpert JS, White HD, Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. Eur Heart J 2007;28:2525–2538; Circulation 2007;116:2634 –2653; J Am Coll Cardiol 2007;50:2173–2195.

18. Thygesen et al. JACC , Expert Consensus Document Month Vol. 60, No. x, 2012 xxx

19. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al; INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004; 364 : 937-52

20. WHO global report 2005 - Preventing chronic diseases: a vital investment. Geneva: World Health Organization; 2005.

21. Vamadevan S. Ajay & Dorairaj Prabhakaran, Coronary heart disease in Indians: Implications of the INTERHEART study Indian J Med Res 132, November 2010, pp 561-566

22. Walldius G, Jungner I. The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy–a review of the evidence. J Intern Med. 2006;259:493–519. [PubMed]

23. Barter PJ. Hugh Sinclair lecture: the regulation and remodelling of HDL by plasma factors. Atheroscler Suppl. 2002;3:39–47. [PubMed]

24. Medical biochemistry John W.Baynes –third edition page no:225-230, Page no:236-240

25. LIPPINCOTT′S illustrated reviews 5 th edition page no:234-236

26. Lusis AJ. Atherosclerosis. Nature. 2000;407:233-241.

27. Glass CK, Witztum JL. Atherosclerosis. the road ahead. Cell. 2001;104:503-516.

28. Libby P. Inflammation in atherosclerosis. Nature. 2002;420:868-874.

29. Suraksha Agrawal and Sarabjit Mastana Genetics of coronary heart disease with reference to ApoAI-CIII-AIVgene region World J Cardiol.2014 AUG 26;6(8):755-767.

30. Enas EA, Singh V, Munjal YP, Bhandari S, Yadave RD, Manchanda SC. Reducing the burden of coronary artery disease in India: challenges and opportunities. Indian Heart J. 2008;60:161-175.

31. Coronary Artery Disease (C4D) Genetics Consortium.A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat Genet. 2011;43:339-344

32. Eichenbaum-Voline S, Olivier M, Jones EL, Naoumova RP, Jones B, Gau B, Patel HN, Seed M, Betteridge DJ, Galton DJ. Linkage and association between distinct variants of the APOA1/C3/A4/A5 gene cluster and familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol. 2004;24:167-174

33. Chien KL, Chen MF, Hsu HC, Su TC, Chang WT, Lee CM, Lee YT. Genetic association study of APOA1/C3/A4/A5 gene cluster and haplotypes on triglyceride and HDL cholesterol in a community-based population. Clin Chim Acta. 2008;388:78-83

34. Delgado-Lista J, Perez-Jimenez F, Ruano J, Perez-Martinez P, Fuentes F, Criado-Garcia J, Parnell LD, Garcia-Rios A, Ordovas JM, Lopez-Miranda J. Effects of variations in the APOA1/C3/A4/A5 gene cluster on different parameters of postprandial lipid metabolism in healthy young men. J Lipid Res. 2010;51:63-73

35. Yin RX, Li YY, Lai CQ. Apolipoprotein A1/C3/A5 haplotypes and serum lipid levels. Lipids Health Dis. 2011;10:140.

36. National centre for biotechnology information –glossary

37. ROBIN'S Textbook of Pathology Chapter 11 pg no: 484-488

38. Kawahiri M, Maugeais C, Rader D: High-density lipoprotein metabolism: molecular targets for new therapies for atherosclerosis. Curr Atheroscler Rep2 :363 –372,2000

39. SoTiRios K. KARATHANASIS, VASSILIS I. ZANNIS, AND JAN L. BRESLOW Isolation and characterization of the human apolipoprotein A-I. gene (preproapolipoprotein A-I/apolipoprotein gene evolution/lipoprotein/rapid M13 replicative form preparation) Natl. Acad. Sci. USA Vol. 80, pp. 6147-6151, October 1983 Biochemistry

40. Oram, J. F., Albers, J. J., Cheung, M. C. & Bierman, E. L. (1981) J. Biol. Chem. 256, 8348-8356.

41. Barker, W C. & Dayhoff, M. 0. (1977) Comp. Biochem. Physiol. B57, 309-315

42. Atherosclerosis, hypertension and diabetes chapter 4

43. Vu-Dac N, Gervois P, Torra IP, Fruchart JC, Kosykh V, Kooistra T, Princen HM, Dallongeville J, Staels B.Retinoids increase human apo C-III expression at the transcriptional level via the retinoid X receptor. Contribution to the hypertriglyceridemic action of retinoids.J Clin Invest. 1998 Aug 1;102(3):625-32.

44. Vu-Dac N, Schoonjans K, Kosykh V, Dallongeville J, Fruchart JC, Staels B, Auwerx J. Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor.J Clin Invest. 1995 Aug;96(2):741-50.

45. Vu-Dac N, Schoonjans K, Laine B, Fruchart JC, Auwerx J, Staels B. Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element.J Biol Chem. 1994 Dec 9;269(49):31012-8.

46. Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nat Med. 2001 Jan;7(1):48-52

47. Bowen NJ, Fujita N, Kajita M, Wade, PA. Mi-2/NuRD: Multiple complexes for many purposes. Biochimica Et Biophysica Acta, 1677(1-3), 200452-57.

48. The expression of the human apolipoprotein genes and their regulation by PPARs

49. XueY, Wong J, Moreno GT, Young MK,Cote J,Wang W. NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Molecular Cell, 2(6), 1998, 851-861.

50. Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev. 2000 Jan 15;14(2):121-41.

51. Lewis BA,ReinbergD. The mediator coactivator complex: Functional and physical roles in transcriptional regulation. Journal of Cell Science, 116(Pt 18), 2003, 3667-3675

52. Miller GJ, Miller NE. Plasma high density lipoprotein concentration and development of ischaemic heart disease. Lancet. 1975;1:16–19

53. Folkertsma S, van Noort P, Van Durme J, Joosten HJ, Bettler E, Fleuren W, Oliveira L, Horn F, de Vlieg J, Vriend G. A family-based approach reveals the function of residues in the nuclear receptor ligand-binding domain. J Mol Biol. 2004 Aug 6;341(2):321-35

54. Shaffer PL, Gewirth DT. Structural basis of VDR-DNA interactions on direct repeat response elements.EMBO J. 2002 May 1;21(9):2242-52

55. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev. 1999 Oct;20(5):649-88

56. Arshag D. Mooradian,1 Michael J. Haas,1 and Norman C.W. Wong

57. G M Hargrove, A Junco and N C W Wong Hormonal regulation of apolipoprotein AI Journal of Molecular Endocrinology (1999) 22, 103–111 0952–5041/99/022–10

58. Douglas C. Harnish, Sohail Malik, EdwardKilbourne, Robert Costa and Sotirios K. Karathanasis Control of Apolipoprotein AI Gene expression through synergistic interaction between Hepatocyte Nuclear Factor 3 and 4J. Biol. Chem. 1996, 271:13621-13628.

59. Samson SL, Wong NC: Role of Sp1 in insulin regulation of gene expression.J Mol Endocrinol 29:265–279, 2002

60. Zheng XL, Matsubara S, Diao C, Hollenberg MD, Wong NC: Epidermal growth factor induction of apolipoprotein A-I is mediated by the Ras-MAP kinase cascade and Sp1. J Biol Chem 276:13822–13829, 2001

61. Geofreyy s.ginsforth,josef ozer,sotirios.S.Karanthanasis Intestinal Apo A1 gene transcription is regulated by multiple distinct DNA elements and is synergistically activated by orphan nuclear receptor Hepatocyte Nuclear Factor-4 ,j.clin.inves, volume 96,july 1995,528-538

62. Lopez-Candales A: Metabolic syndrome X: a comprehensive review of the pathophysiology and recommended therapy. J Med 32:283–300, 2001

63. Vajo Z, Terry JG, Brinton EA: Increased intra-abdominal fat may lower HDL levels by increasing the fractional catabolic rate of Lp A-I in postmenopausal women. Atherosclerosis 160:495–501, 2002

64. Duvillard L, Pont F, Florentin E, Gambert P, Verges B: Inefficiency of insulin therapy to correct apolipoprotein A-I metabolic abnormalities in non-insulin-dependent diabetes mellitus. Atherosclerosis 152:229–237,2000

65. Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Jean-Louis Chiasson J-L, Garg A, Holzmeister LA, Hoogwerf B, Mayer-Davis E, Mooradian AD, Purnell JQ, Wheeler M: Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications (Technical Review). Diabetes Care 25:148–198, 2002

66. Elizabeth G. Nabel, M.D., and Eugene Braunwald, M.D. A Tale of Coronary Artery Disease and Myocardial Infarction T h e new engl and journal o f medicine N Engl J Med 2012;366:54-63.

67. Godfrey S Getz Catherine A Reardon Apolipoprotein A-I and A-I mimetic peptides:a role in atherosclerosis Journal of Inflammation Research 2011:4

68. Carla cuthbert,zhiwei wang,Xia zhang and shui pang Tam Regulation of Human Apolipoprotein A-I Gene Expression by Gramoxone J. Biol. Chem. 1997 272: 14954-14960. doi:10.1074/jbc.272.23.14954

69. Ajees, A. A., Anantharamaiah, G. M., Mishra, V. K., Hussain, M. M., Murthy, H. M. K. Crystal structure of human apolipoprotein A-I: insights into its protective effect against cardiovascular diseases. Proc. Nat. Acad. Sci. 103: 2126-2131, 2006

70. Fryirs MA, Barter PJ, Appavoo M, et al. Effects of high-density lipoproteins on pancreatic beta-cell insulin secretion. Arterioscler Thromb Vasc Biol. 2010;30:1642–1628.

71. W.Seandavidson& thomas . R.Thompson The Structure of Apolipoprotein A-I in High Density LipoproteinsAugust 3, 2007 The Journal of Biological Chemistry, 282, 22249-22253.

72. Philippe G.Frank , yves.v.marcel Apolipoprotein A-I: structure–function relationships June 2000 The Journal of Lipid Research, 41, 853-872.

73. A. Abdul Ajees*, G. M. Anantharamaiah†, Vinod K. Mishra†, M. Mahmood Hussain‡, and H. M. Krishna Murthy*§ Crystal structure of human apolipoprotein A-I:Insights into its protective effect againstcardiovascular diseases

74. Ruth Shemer, Annemarie Walsh+, Shlomo Eisenbergf, Jan L. BreslowS, and Aharon RazinY Tissue-specific Methylation Patterns and Expression of the Human Apolipoprotein AI Gene* THE JOURNAL. OF BIOLOGICAL. CHEMISTRY Vol. 265, No. 2, Issue of January 15, pp. lOlO-1015,199O

75. Dmitri sviridov, Anh Hoang,Wei Huang and Jun SASA Structure-function studies of apoA-I variants site-directed mutagenesis and natural mutations

76. Xi-Long Zheng, Shuji Matsubara, Catherine Diao, Morley D. Hollenberg and Norman C. W. Wong Activation of Apolipoprotein AI Gene Expression by Protein Kinase A and Kinase C through Transcription Factor, Sp1 J. Biol. Chem. 2000, 275:31747-31754.

77. Nicole Y. Tan and Levon M. Khachigian Sp1 Phosphorylation and Its Regulation of Gene Transcription. molecular and cellular biology,may 2009 ,page 2483-2488

78. Philip J. Barter, H. Bryan Brewer Jr, M. John Chapman, Charles H. Hennekens, Daniel J. Rader,Alan R. TallCholesteryl Ester Transfer ProteinA Novel Target for Raising HDL and Inhibiting Atherosclerosis

79. Beneficial Effects of Fibrates on Apolipoprotein A-I Metabolism Occur Independently of Any Peroxisome Proliferative Response Nathalie Hennuyer, BS; Philippe Poulain, BS; ,Lise Madsen, BS;, Rolf K. Berge, PhD; Louis-Marie Houdebine, PhD; Didier Branellec, PhD; Jean-Charles Fruchart, PhD; Catherine Fiévet, PhD; Nicolas Duverger, PhD; Bart Staels, PhD

80. X.L. Wang, S.-X. Liu, R.M. McCredie, and D.E.L. Wilcken Polymorphisms at the 5'End of the Apolipoprotein AI Gene and Severity of Coronary Artery Disease Volume 98, Number 2, July 1996, 372–377

81. Rajni Dawar, MD,1 Anil Gurtoo, MD,2 and Ritu Singh, MD1 Apolipoprotein A1 Gene Polymorphism (G–75A and C+83T) in Patients With Myocardial Infarction A Pilot Study in a North Indian Population Am J Clin Pathol 2010;134:249-255

82. Packard CJ. Apolipoproteins: the new prognostic indicator? Eur Heart J Suppl. 2003;5:D9-D16

83. Luciana Moreira Lima; Maria das Graças Carvalho; Marinez Oliveira Sousa Apo B/apo A1ratio and cardiovascular risk prediction Arq. Bras. Cardiol. vol.88 no.6 São Paulo June 2007

84. Smith JD, Brinton EA, Breslow JL. Polymorphism in the human apolipoprotein AI gene promoter region: association of the minor allele with decreased production rate in vivo and promoter activity in vitro. J Clin Invest. 1992;89:1796-1800.

85. Reguero JR, Cubero GI, Batalia A. Apolipoprotein A1 gene polymorphism and risk of early coronary disease. Cardiology.1998;90:231-235

86. Padmaja, M.R. Kumar, C. Adithan. "Association of polymorphisms in apolipoprotein A1 and apolipoprotein B genes with lipid profile in Tamilian population." Indian Heart J., vol. 61, pp. 51-54, 2009.

87. S. Chhabra, R. Narang, R. Lakshmy, N. Das. "APOA1-75 G to A substitution associated with severe forms of CAD, lower levels of HDL and apo-A1 among northern Indians." Dis Markers, vol. 21, pp. 169-74, 2005

88. Tanzeela Khana, Falak Qazib, Sabha Rasoolc, Samia Rashidd, Qulsum Akhtare, Akbar Masoodf and Bashir Ahmad Ganaig* Genetic Variants at the Apo-A1 Gene in Association with Coronary Artery Disease International Journal of Sciences: Basic and Applied Research (IJSBAR) (2012)Volume 5, No 1, pp33-42

89. Wang XL, Badenhop R, Humphrey K, et al. New MspI polymorphism at +83 bp of the human apolipoprotein A1gene: association with increased circulating high density lipoprotein cholesterol levels. Genet Epidemiol. 1996;13:1-10.

90. Angotti E, Mele E, Costanzo F, et al. A polymorphism(G-A transition) in the –78 position of the apolipoprotein A-I promoter increases transcription efficiency. J Biol Chem.1994;26:17371-17374.

91. F. Pagani,' A. Sidoli,* G. A. Giudici, L. Barenghi, C. Vergani,t and F. E. Baralle.Human apolipoprotein A-I gene promoter polymorphism: association withhyperalpha1ipoproteinemia Journal of Lipid Research Volume 31, 1990

92. Bahar toptas, uzay gormuş, arzu ergen1, hakan gurkan, fatih keleşoğlu,feyza darendeliler, firdevs bas, altay burak dalan, guldal izbirak and turgay isbirComparison of Lipid Profiles with APOA1 MspI Polymorphism in Obese Children with Hyperlipidemiain vivo 25: 425-430 (2011)

93. National health statistics provided by Member States), or the latest *GlobalBurden of Disease: 2004 Update* data (also based on WHO country data) (2008a)

94. ARTO PULKKINEN, MD ,LAURA VIITANEN, MD,ANU KAREINEN, MD,SEPPO LEHTO, MD,MARKKU LAAKSO, MDMspI polymorphism at +83 bp in intron 1 of the human apolipoprotein A1 gene is associated with elevated levels of HDL cholesterol and apolipoprotein A1 in nondiabetic subjects but not in type 2 diabetic patients with coronary heart disease. DIABETES CARE, VOLUME 23, NUMBER 6, JUNE 2000

95. E. de França1,J.G.B. Alves2 and M.H. Hutz1 APOA1/C3/A4 gene cluster variabilityand lipid levels in Brazilian children Brazilian Journal of Medical and Biological Research (2005) 38: 535-541.